VOLUME 18

STUDY TITLE

Waiver Request – Nontarget Organisms:
Capric Acid

DATA REQUIREMENTS

850.1010: Freshwater invertebrate LC50
850.1075: Fish acute toxicity, freshwater
850.2100: Avian acute oral
850.2200: Avian dietary LC50
850.4100: Seedling emergence
850.4150: Vegetative vigor
880.4350: Nontarget insect testing

COMPLETION DATE

March 27, 2013

COMPILED BY

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SPONSOR

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STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality, on any basis whatsoever, is made for any information contained in this document. I acknowledge that information not designated as within the scope of FIFRA § 10(d)(1)(A), (B) or (C) and which pertains to a registered or previously registered pesticide is not entitled to confidential treatment and may be released to the public, subject to the provisions regarding disclosure to multinational entities under FIFRA § 10(g).

Company: Westbridge Agricultural Products

Company Agent: Frederick T. Smith

Title: Agent*

Signature:

Date: 3/27/13

*SciReg, Inc. is the authorized agent for Westbridge Agricultural Products

GOOD LABORATORY PRACTICES COMPLIANCE STATEMENT

The material presented in this section is not a study but a presentation of factual information and is, therefore, not subject to GLP requirements. This report is a compilation of technical information and it did not have a Study Director.

Sponsor/Submitter: _____

Date: 3/29/13

BioLink® Herbicide
Justification for Waiver of the Biochemical Eco-Toxicological Data Requirements:

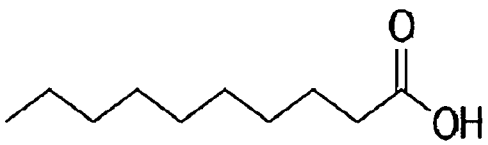
Avian acute oral (850.2100)
Avian dietary LC50 - duck (850.2200)
Avian dietary LC50 - quail (850.2200)
Fish acute toxicity, freshwater (850.1075)
Freshwater invertebrate LC50 (850.1010)
Seedling emergence (850.4100)
Vegetative vigor (850.4150)
Nontarget insect testing (850.4350)

Overview

BioLink® Herbicide, hereafter referred to as the Herbicide, is a product being submitted to the EPA for registration in the United States. The Herbicide is a non-selective, post-emergent, contact material that will be labeled for use against various broadleaf and grass annuals and perennials, as well as to control sucker growth on trees, and to thin blossoms on pome trees. Direct application to growing crops will not occur; incidental exposure, however, is possible. The active ingredients in the Herbicide are capric acid (syn. decanoic acid; CAS# 334-48-5) and caprylic acid (syn. octanoic acid; CAS# 124-07-2). The Technical Grade Active Ingredient (TGAI) for the product is a blend of caprylic and capric acids at 59.49% and 40.47%, respectively. These are free fatty acids fractionated from coconut and/or palm oil extracts. The fatty acids are removed from the glycerol backbone through steam hydrolysis and fractionally distilled to yield the specific fatty acids of interest. The Herbicide will be marketed as a concentrate with caprylic and capric acids at 47.0% and 32.0%, respectively. The label specifies end-use rates of 3-9% with carrier volumes of 40-100 gallons per acre (GPA). The mode of action for this Herbicide is as a desiccant through the disruption of cuticular integrity and cell membranes, as well as certain physiological functions. Once cell membranes are compromised, cellular leakage and death follow (1). Label language stating to spray until wet and minimize run-off will help keep vertical drift contained and, with the significant possibility of crop damage, horizontal drift will be contained by the grower. In addition, many uses of the Herbicide will be target-directed spot applications. The purpose of this paper is to serve as a justification for waiver of the eco-toxicity data requirements. While capric and caprylic acids are new actives in regard to herbicide use, they are not new when used as antimicrobial active ingredients. Many of the properties and reactions of numerous fatty acids have been determined in previous evaluations and experiments. The paper will progress from a generalized discussion of fatty acid chemistry and relations to a summary of the properties of capric acid, in particular. In addition, a specific precedence will be detailed in which a waiver of eco-toxicological data requirements had been granted by EPA for an active ingredient having a very similar eco-toxicological profile.

Lipids occur naturally in a number of biologically active forms including fatty acids, phospholipids, lipoproteins, sphingolipids, sterols, and glycolipids (2,3). Fatty acids, as a chemical class, are ubiquitous

in nature and occur in virtually all life forms. Fatty acids will typically occur as an acylglyceride or phospholipid, but they also occur in a free, or unbound, form. Acylglycerides are often a "storage" form of fatty acids since their metabolism has to be preceded by the fatty acids released from the glycerin backbone via hydrolysis reactions. Frequently, the acylglyceride is in the form of a triacylglyceride which is a glycerol "backbone" with three fatty acids esterified to the glycerol, although mono- and diacylglycerides occur. The fatty acids in a triacylglyceride are usually different from each other. Structurally, fatty acids are an aliphatic alkyl functional group with at least one terminal carboxylic acid functional group. Aliphatic alkyl functional groups are straight chain hydrocarbons, either saturated or unsaturated. A saturated fatty acid has all the carbons in the alkyl functional group "saturated" with hydrogen atoms. An unsaturated fatty acid has at least one bond which is a double bond. The carbon atoms on both sides of a double bond have one less hydrogen than a saturated carbon would have. Usually the alkyl chains have an even number of carbons in length, although there are exceptions (2,3). The following structures depict capric and caprylic acids.



Capric Acid

Fatty acids are considered amphiphilic; they have hydrophilic and hydrophobic domains within the same molecule. The carboxylic acid functional group (right side in the figures) is the hydrophilic end of the molecule. This is the "business end" of the molecule. The chemically active sites for fatty acid reactions and metabolism are the carboxylic acid functional group and any double or triple bonds that may be present. This is because many chemical reactions are nucleophilic or electrophilic substitution reactions. These reactions require asymmetry in electrical charge distribution which occurs around the carboxylate functional group and unsaturated carbon bonds in the alkyl functional group. For example, synthesis and degradation processes will typically cleave two carbons at a time from the carboxylic acid functional group end(s) of the fatty acid (2,3). As can be seen above, capric and caprylic acids have no double bonds, they are saturated.

The carboxylic acid functional group is what makes fatty acids an acid, and as such it can be either the protonated acid or the unprotonated conjugate base form (anion). The form a fatty acid takes is a function of the environmental pH in which it exists and that specific fatty acid's pKa. If the pH is above the pKa, then it is in anionic form and if it is below the pKa then it is in a protonated, or acid form. The pKa of a fatty acid decreases slightly with decreasing carbon chain length of the alkyl functional group (2,3,4,5). Typical pKa's for medium chain length saturated fatty acids are at or slightly less than five (5).

Fatty acids are readily and rapidly degraded in the environment as evidenced by their relative lack of accumulation despite constant, appreciable inputs. Lipids in general, and fatty acids in particular, are ubiquitous in all life forms. They exist in free forms and bound forms, such as sphingolipids, phospholipids, and acylglyderides. These can be distributed through various tissues such as cuticle, membranes, and suberin (2,3). The environmental inputs are constant and substantial. As is true with most molecular breakdown processes, the remediation of fatty acids can be separated into biotic and abiotic degradation routes.

Abiotic degradation is accomplished without the activity of biological organisms, while biotic degradation involves the activity of biological organisms. Photolysis and hydrolysis are common abiotic degradation reactions, although with a saturated fatty acid such as capric acid, abiotic degradation is not common due to a lack of reactive sites on the molecules (2,3). Assimilation, either intercellularly or extracellularly, are the primary biotic degradation routes. Fatty acids are assimilated nutritionally by most animals, plants, and microbes as both an energy (ATP) and carbon source (2,3). They are an important part of the human diet (9). The prevalent degradation of fatty acids occurs by the removal of two carbon atoms at a time. Due to the sequential cleaving of two carbons, the intermediate metabolites of a fatty acid are often, a shorter fatty acid. Ultimately, carbon dioxide and water are the breakdown products of all fatty acids. Within the fatty acids, relative degradation rates increase as the length of the fatty acid increases, as well as the degree of unsaturation (2,3).

Capric acid is a saturated fatty acid having ten carbons. It is considered a medium-chain, semi-volatile fatty acid (2,3,11). Capric acid has a history in the flavorings and cleaning industries. Biological sources for capric acid include apples, beer, preferments of bread, butter, cheese, blue cheese, Romano cheese, Roquefort cheese, roasted cocoa bean, cognac, Muscat grape, grape musts and wine, and other natural sources. In addition, it has also been reported in citrus peel oils, orange juice, apricots, guava, papaya, strawberry, yogurt, milk, mutton, hop oil, bourbon, and scotch whiskey, rum, coffee, mango, and tea (8). In addition, capric acid is common in mammal milk, and at certain times, human milk (8). While capric acid may occur at a relatively low level compared to long-chain fatty acids, there are still a still number of natural occurrences documented. In addition, *Cuphea koehneana* seed oil is composed of almost 96% capric acid. This is one of the highest purities for any known naturally occurring fatty acid. Several other Cuphea species have over 90% of their total seed oil comprised of capric and caprylic acids (8,10). Even so, industrially, capric acid is primarily derived from coconut and palm plant species.

Fatty Acid Metabolism

Fatty acids have a number of uses ranging from nutritional and cosmetic to agricultural. The human body cannot synthesize two fatty acids that are needed as precursors to several key metabolites. These are termed essential fatty acids and are, specifically, linoleic acid and alpha-linoleic acid (9). Both have to be included in the diet, but are present in numerous plant and fish species. Triacylglycerides are traditionally a storage form of fatty acids and as such are transported to different parts of the body for future use, as needed. Free fatty acids of short and medium chain length are directly absorbed through the intestinal lining, while the free long-chain fatty acids are absorbed by the villi and formed into

chlyomicrons, a combination of fatty acids, protein, and cholesterol, which are then transported to the heart for further distribution. Ultimately, regardless of origin, the breakdown of the fatty acids occurs in the mitochondria and is accomplished via beta oxidation (2,3,9,11). Beta oxidation is the sequential cleavage of two terminal carbons at the carboxylate end of the fatty acid. As such, the metabolites of fatty acid degradation are sequentially shorter chain fatty acids, ultimately resulting in a single two-carbon fragment.

Agricultural Fatty Acid Uses

Traditionally, one of the primary uses for fatty acids is to make soaps. The soaps produced can be cosmetic or pesticidal in use, as is the case of horticultural soaps. Triacylglycerides are converted to glycerol and fatty acids by hydrolysis of the ester bond connecting the two constituents (2,3,11). This can be accomplished in one step by the use of hard bases, or lye (NaOH or KOH) or in two steps using steam to hydrolyze the fatty acid then neutralizing with a base to form the salt (6,11). The neutralized salts of fatty acids are soaps (6). Hard soap is associated with a sodium cation and soft soap has a potassium cation. This process is termed saponification (2,3,11). In addition, soap can have other cations such as copper. In this case, the copper is added as copper sulfate to the anionic fatty acid solution to serve as the cation. Horticultural soaps, while in solution, are mixtures of cations (potassium, sodium, copper, etc.) and the anionic fatty acid. Direct association of the cation with the anion does not occur prior to the removal of water, and only then if the cation is not preferentially scavenged by a stronger anion (11).

Physical and Chemical Properties of Capric and Caprylic Acids

Fatty acids have been extensively studied for many years. Their roles in making soaps and cosmetics, as well as their nutritional implications, have driven many of these studies. As such, there is data available on numerous individual fatty acids' toxicological, chemical and physical properties. In addition, several fatty acids and their derivatives have been registered as pesticide active ingredients and have undergone the required tests for registration purposes. Some examples are the numerous potassium salts of fatty acids, copper soap, and a fatty acid based herbicide.

Capric and caprylic acids are ten and eight carbon fatty acids, respectively. Both are considered medium chain length, semi-volatile fatty acids. Because of their structural and functional similarities, they could be considered as adjacent points in a gradient of values, in respect to many of their chemical and physical properties. As such, capric and caprylic acids have very similar pKas, octanol-water partition coefficients, water solubilities, and other related properties. Table 1 summarizes some of these properties and their respective values (10,11,12,18). Pelargonic acid, another saturated fatty acid intermediate in length with capric and caprylic acids, is included for comparison purposes. Pelargonic acid will be discussed further in this paper.

Table 1. Some chemical and physical properties of select fatty acids

Property	Caprylic Acid	Pelargonic Acid	Capric Acid
CASRN	124-07-2	112-05-0	334-48-5
Carbon Chain Length	**8**	**9**	**10**
log Kow	3.05	3.42	4.09
Koc (estimated)	1100	1700	4000
pKa	4.89	4.95	4.90
Vapor pressure (mm Hg@25 C)	3.71e-3	1.65e-3	3.66e-4
Specific Gravity (g/cc)	0.9088	0.9057	0.8858
Melting Point (°C)	16.5	12.24	31.5
Boiling Point (°C)	239.0	254.5	268.7
Heat of Combustion (kJ/mol)	-4799.9	-5456.1	-6108.7
Surface Tension (mN/m@70 C)(=dyn/cm)	23.7	N/A	25.0

Environmental Fate of Capric Acid

There are numerous studies regarding the environmental fate of capric acid. In aerobic environments virtually all microorganisms can metabolically degrade fatty acids (2,3). Overall, the persistence of fatty acids in the environment is fairly short because of the wide spread occurrence of the numerous organisms capable of metabolizing these structures. Ultimately, the degradation of fatty acids occurs in cell mitochondria via beta oxidation.

The log Kow's for capric acid is 4.09 (10,12). A larger value is more hydrophobic and this value indicates a strong preference for a hydrophobic (octanol) phase compared to a hydrophilic (water) phase.

The Koc value is 4000 for capric acid (18). The Koc value is an equilibrium constant for organic carbon-water partitioning. These are distribution constants normalized for soil organic carbon content. The value is positively correlated to the amount of compound adsorbed by the soil, per unit soil organic carbon. Higher values indicate less soil mobility, since soil adsorption is inversely proportional to water solubility (18). These are calculated, or predicted, values using primarily Kow's and they indicate that these fatty acids would be expected to have very little mobility in the soil. In addition, it would be predicted that the potential for groundwater or runoff water is relatively low (18).

There are limited studies of fatty acid persistence in water. It has to be kept in mind that these are sparingly soluble in water, at best. Japanese studies of capric acid in fresh and salt water showed 100% degradation within 3 days (13). The shorter chained caprylic acid could be expected to degrade in the same time frame, if not shorter, due to its truncated chain length.

The pKa for capric acid is 4.90 (5,12,14,18). Based on soil pH tendencies and its buffering capacity, any residues of capric acid would be expected to be anionic in form (18). Since the soil clay fraction

and organic matter are negatively charged, electrostatic interactions with charged soil solids are highly unfavorable. Volatilization from moist soils would not be anticipated due to the charge associated with the residues (18).

Capric acid has a vapor pressure of 3.71x1066X10-4 mm Hg (12,18). This low vapor pressure indicates that in the air this fatty acid would exist as a vapor only. Vapor-phase capric acid would be degraded by photochemically produced hydroxyl radicals and the estimated half-life is 1.4 days (19). Significant volatilization from dry soils is not anticipated due to the low associated vapor pressure of capric acid (12,18).

Most soil microbial populations can readily degrade fatty acids. This is because most organisms utilize fatty acids as a source of energy and carbon (2,3). As with metabolism within the human body, beta-oxidation is the manner in which fatty acids are degraded by microorganisms. The carboxylic acid is reacted with coenzyme A, which replaces the alcohol functional group of the carboxylic acid with a thiol ester. After this "priming," two carbons at a time are cleaved until acyl-CoA remains (2,3). Fatty acid half-lives, based on BOD (Biological Oxygen Demand), are variable, probably due to differences in the associated microbial populations. Nonetheless, in several experiments, degradation of 50% of the added fatty acid occurred from several hours to 20 days after inoculation with microbes (12,18).

Overall, there is little chance for groundwater or runoff contamination, vapor-phase accumulation, or soil persistence from these materials in the form that they would exist and the quantities in which they would be present as in the ecosystem.

But perhaps the most compelling argument for waiving the eco-toxicity requirements for capric acid applied to weeds in and around growing agricultural crops is the precedence set with the registration of Scythe Herbicide (Mycogen Corporation; EPA Reg. No. 53219-7; transferred to Gowan Company under EPA Reg. No. 10163-325), a fatty acid-based herbicide marketed for the same uses as those proposed for BioLink Herbicide (1). The active ingredient in Scythe is pelargonic (nonanoic) acid, a nine-carbon, saturated, fatty acid which was granted a waiver from the eco-toxicity requirements by EPA. Pelargonic acid is intermediate in length to capric and caprylic acids and so its toxicological, as well as physical and chemical properties, would be expected to be very similar to capric and caprylic acids. In addition, the normal environmental exposure levels to pelargonic acid are much less than capric and caprylic acids just on the basis of it being a nine-carbon fatty acid.

Summary and Conclusions

Fatty acids, including capric acid, are completely biodegradable and ubiquitous in nature. They are common constituents of virtually all life forms. Capric acid, being a saturated fatty acid, rarely undergoes abiotic hydrolytic reactions due to the lack of reactive sites within the molecules. However, they are readily metabolized and consumed by all life forms including man. In the soil they are readily metabolized by microorganisms.

The best direct comparison to a registered product is Scythe Herbicide. Even though Scythe is based on an unusual carbon length fatty acid that is relatively uncommon in nature, EPA nonetheless waived the eco-toxicity data requirements. Capric and caprylic acids both compare favorably to pelargonic acid in respect to environmental impact and potential toxicological implications.

The form in which the active ingredients of the Herbicide will exist after their application has minimal potential for groundwater or runoff contamination, volatilization, or rhizosphere persistence. These compounds are a ubiquitous organic input and as such do not introduce an exotic, or xenobiotic, agent into the ecosystem. Mechanisms for their degradation and metabolism are already in place.

Horticultural soaps have been used for decades for insect control, and to a lesser extent, plant disease control. These materials are salts of fatty acids and therefore the solution phase active ingredient is the fatty acid anion, or conjugate base. The environmental fate of these soaps is the same as the environmental fate of the conjugate base of their respective fatty acid.

Capric acid, one of the two active ingredients for the Herbicide, is a new active for biochemical pesticide registration. In spite of this, it should not be considered novel, since there is a wealth of information on fatty acids in general, as well as this one in particular. Because the structure of a fatty acid is predominantly a carbon chain of variable length, their metabolites are very similar to the parent material, just differing in the length of the carbon chain and functional groups present. Fatty acids have been extensively studied as nutritional components, cleaning agents, food products, and pharmacological active ingredients.

Since the proposed product is an herbicide, the target of the applications will be weeds associated with crop plants, not the actual crop plants. It is not the same situation as an insecticide or fungicide which would be applied directly to a crop. If a crop is inadvertently sprayed, it would not be marketable. Growers are aware of this and the potential for income loss is one of the best deterrents to misuse of an herbicide. With side shields routinely used by the grower and with the label language stating to apply until wet, not until run-off, the potential for crop contamination and drift is minimal. Even so, due to the preponderance of biological organisms able to readily assimilate the fatty acids, as well as the low potential for groundwater contamination and volatilization, there is minimal risk for environmental contamination with the Herbicide.

Finally, EPA has waived all of the ecotoxicity data requirements for capric acid, based on its low toxicity, biodegradable nature, and the fact that it is readily metabolized by all forms of life.

BioLink® Herbicide Bibliography

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REFERENCE 1

[Federal Register Volume 62, Number 16 (Friday, January 24, 1997)]
[Notices]
[Pages 3688-3691]
From the Federal Register Online via the Government Printing Office [www.gpo.gov]
[FR Doc No: 97-1753]

ENVIRONMENTAL PROTECTION AGENCY
[PF-685; FRL-5579-3]

Mycogen Corporation; Pesticide Tolerance Petition Filing

AGENCY: Environmental Protection Agency (EPA).

ACTION: Notice of filing.

SUMMARY: This notice announces the filing of a pesticide petition proposing a regulation establishing an exemption from the requirement of a tolerance for residues of the pesticide pelargonic acid on all raw agricultural commodities. This notice includes a summary of the petition that was prepared by the petitioner, Mycogen Corporation.

DATES: Comments, identified by the docket control number [PF-685], must be received by EPA on or before February 24, 1997.

ADDRESSES: By mail, submit written comments to: Public Response and Program Resources Branch, Field Operations Division (7506C), Office of Pesticide Programs, Environmental Protection Agency, 401 M St., SW., Washington, DC 20460. In person, bring comments to: Rm. 1132, Crystal Mall #2, 1921 Jefferson Davis Hwy., Arlington, VA.

Comments and data may also be submitted electronically by sending electronic mail (e-mail) to: opp-docket@epamail.epa.gov. Electronic comments must be submitted as an ASCII file avoiding the use of special characters and any form of encryption. Comments and data will also be accepted on disks in WordPerfect 5.1 file format or ASCII file format. All comments and data in electronic form must be identified by docket number [PF-685]. No ``Confidential Business Information'' (CBI) should be submitted through e-mail. Electronic comments on this notice of filing may be filed online at many Federal Depository Libraries. Additional information on electronic submissions can be found in Unit II. of this document.

Information submitted as a comment concerning this document may be claimed confidential by marking any part or all of that information as CBI. Information so marked will not be disclosed except in accordance with procedures set forth in 40 CFR part 2. A copy of the comment that does not contain CBI must be submitted for inclusion in the public record. Information not marked confidential may be disclosed publicly by EPA without prior notice. All written comments will be available for public inspection in Rm. 1132 at the address given above, from 8:30 a.m. to 4 p.m., Monday through Friday, excluding legal holidays.

FOR FURTHER INFORMATION CONTACT: Michael Mendelsohn, Biopesticides and Pollution Prevention Division (7501W), Office of Pesticide Programs, Environmental Protection Agency, 401 M St., SW., Washington, DC 20460. Office location, telephone number, and e-mail address: 5th Floor, CS #1, 2805 Jefferson Davis Highway, Arlington, VA, 703-308-8715; e-mail: mendelsohn.michael@epamail.epa.gov.

SUPPLEMENTARY INFORMATION: EPA has received pesticide petition (PP) 6F4625 from Mycogen Corporation, 4980 Carroll Canyon Road, San Diego, CA 92121. The petition proposes, pursuant to section 408 of the Federal Food, Drug, and Cosmetic Act (FFDCA), 21 U.S.C. 346a, to amend 40 CFR part 180 by establishing an exemption from the requirement of a tolerance for residues of pelargonic acid on all raw agricultural commodities. EPA has determined that the petition contains data or information regarding the elements set forth in section 408(d)(2); however, EPA has not fully evaluated the sufficiency of the submitted data at this time or whether the data support granting of the petition. Additional data may be needed before EPA rules on the petition.

Mycogen has stated that an analytical method for the detection and measurement of pelargonic acid residues is not necessary to protect the public health and environment. They state that the natural occurrence of pelargonic acid in our food supply and environment, and the rapid metabolism and degradation of pelargonic acid to background levels in humans, plants and soil, eliminate the need to quantify pelargonic acid residues.

As required by section 408(d) of the FFDCA, as recently amended by

the Food Quality Protection Act, Mycogen included in the petition a summary of the petition and authorization for the summary to be published in the Federal Register in a notice of receipt of the petition. The summary represents the views of Mycogen; EPA, as mentioned above, is in the process of evaluating the petition. As required by section 408(d)(3) EPA is including the summary as a part of this notice of filing. EPA may have made minor edits to the summary for the purpose of clarity.

I. Petition Summary

This unit summarizes information cited by Mycogen to support the proposed tolerance.

A. Pelargonic Acid Uses

Pelargonic acid is currently used as the active ingredient in two unique pesticide products. First, it is used as a contact, non-selective, broadspectrum, foliar-applied herbicide. As the active ingredient in Scythe Herbicide (EPA Reg. No. 53219-7), registered by EPA for non-crop uses on April 7, 1994, pelargonic acid will only control actively growing emerged green vegetation. Pelargonic acid provides burndown of both annual and perennial broadleaf and grass weeds, as well as most mosses and other cryptogams. The spray quickly penetrates plant tissue and disrupts normal cell membrane permeability and cellular physiology. The disruption of the cell membrane results in cell leakage and death of all contacted tissue. The product does not translocate, and it will burn only those plant parts that make contact with spray solution. Scythe provides no residual

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weed control; therefore, repeat treatments may be necessary for new plants emerging from seed or regrowth of treated vegetation.

Scythe Herbicide contains as the active ingredient 57 percent pelargonic acid and 3 percent related fatty acids (C6 - C12). One gallon of Scythe contains 4.2 pounds (lbs) of pelargonic acid. The application rate will range from 3 percent to 10 percent v/v delivered at 75 to 200 gallons of spray solution per acre through boom, hand-held, or high volume equipment. Therefore, the rate of use of pelargonic acid will be 9.45 lbs to 84 lbs per acre. Combinations with selected products may further reduce the application rate to a low 0.78 lbs to 2.1 lbs per acre (0.25 percent solution in 75 or 200 gallons spray per acre).

Second, pelargonic acid is used as a fruit blossom thinner that promotes return bloom (annual bearing) and increased fruit size and quality in apple and pear. Thinex Blossom Thinner (EPA Reg. No. 53219-11) was registered as a biochemical pesticide due to the natural occurrence of pelargonic acid, the low use rates and the unique non-toxic mode of action. Thinex works on contact by damaging the stigma or female flower part of the blossom, thus preventing pollination of a certain percentage of flowers. A blossom that has already been fertilized at the time of application will be undamaged by Thinex. No more than 2 applications per year are made. On February 14, 1996, pelargonic acid was exempt under 40 CFR 180.1159 from the requirement of a tolerance when used as a blossom thinning agent on apple and pear.

Thinex Blossom Thinner contains as the active ingredient 57 percent pelargonic acid and 3 percent related fatty acids (C6 - C12). The application rate as a blossom thinner ranges from 0.5 pints to 4 pints of product to make 100 gallons of spray solution. One hundred to 400 gallons of spray solution per acre may be used. Therefore, the rate of use of pelargonic acid as a blossom thinning agent ranges from a low 0.26 lbs to a high 8.4 lbs per acre.

B. Product Identity/Chemistry

Pelargonic acid ($C_8H_{17}COOH$), a nine-carbon straight-chain fatty acid commonly referred to as nonanoic acid, is a naturally-occurring fatty acid found in the environment and in our food supply.

Pelargonic acid has been found to occur naturally in low concentrations in soil. The degradation of pelargonic acid applied to soil occurs very rapidly by microbial means, not through hydrolysis or photolysis. Degradation occurs under aerobic conditions with beta-oxidation being the principal pathway of metabolism.

Pelargonic acid has been shown to occur naturally in our food supply. For example, it has been identified in grapes, cheese and milk at levels from 10 parts per million (ppm) to 400 ppm. Some literature references cite its natural occurrence in soybeans (trace levels), oranges (130 ppm), beans (trace levels), tobacco (0.27 ppm) and potatoes (1.18 ppm). In a cross-section of apple varieties analyzed by Mycogen, pelargonic acid was found at levels from 20 parts per billion

(ppb) to 320 ppb.

Fatty acids, including pelargonic acid, are metabolized in mammalian systems to produce energy. The oxidative degradation of fatty acids is a central metabolic pathway in humans, animals and plants. Fatty acids of varying chain lengths are metabolized into two-carbon fragments through a sequence of enzyme-catalyzed reactions. The metabolic products are then incorporated into fats, carbohydrates and amino acids.

The magnitude of pelargonic acid residues from applications of Scythe Herbicide anticipated at time of harvest will be insignificant beyond naturally-occurring levels and to normal dietary exposure. Applications of Scythe Herbicide will not directly contact desirable food commodities since exposure will be intentionally avoided by the grower because crop damage may result. Any residues of pelargonic acid on food commodities will only occur as a result of spray drift, thus minimizing residues of pelargonic acid on the food commodity.

An analytical method for detecting and measuring the levels of pelargonic acid residue is not necessary to protect the public health and environment. The natural occurrence of pelargonic acid in our food supply and environment, and the rapid metabolism and degradation of pelargonic acid to background levels in humans, plants and soil, eliminate the need to quantify pelargonic acid residue from applications as a herbicide or a blossom thinner.

C. Mammalian Toxicological Profile

Mycogen has submitted to EPA a comprehensive toxicology data package and referenced several published articles concluding that residues of pelargonic acid will be safe to human health.

Although a significant concentration of pelargonic acid can be irritating to eyes and skin, toxicology data confirms that exposure to residues of pelargonic acid beyond naturally occurring background levels will be practically non-toxic to human health. The following mammalian toxicity studies have been conducted to support the tolerance exemption for residues of pelargonic acid:

Acute Oral LD50: >5000 mg/kg
Acute Dermal LD50: >2000 mg/kg
Acute Inhalation LC50: >1.244 mg/L
Dermal Irritation (Rat): Severely Irritating
Eye Irritation (Rabbit): Severely Irritating
Skin Sensitization (Guinea Pig): Not sensitizing

A range finding test to determine dosing concentrations for a 90-Day Rat Oral Toxicity study produced no adverse effects from pelargonic acid at any dose level for 3 weeks, including the highest dose of 20,000 ppm (2 percent), or 1,834 mg/kg/day, for a period of 2 weeks.

A developmental toxicity screen study in rats produced a NOEL of 1,500 mg/kg/day (only dose tested). Pelargonic acid was tested at one dose administered by gavage in corn oil to 22 CD rats (20 pregnant) on days 6 through 15 of gestation. No evidence of maternal or developmental toxicity was seen.

A chronic dermal toxicity study in mice resulted in no evidence of severe dermal or systemic toxicity. Fifty mice were treated twice-weekly with 50 mg doses of undiluted pelargonic acid for 80 weeks. Histopathology revealed no tumors of the skin or the internal organs.

A gene mutation assay in mouse lymphoma cells (L5178Y TK) concluded that pelargonic acid was negative for inducing mutations without metabolic activation, and was considered weakly positive for inducing mutations at the TK locus of culture mouse (L5178Y TK) cells in the presence of S9-induced metabolic activation. Mutations were induced at levels greater than or equal to 50 mg/ml. However, this occurred in the presence of increasing moderate-to-severe cytotoxicity and small colony development and may reflect gross chromosomal changes or damage rather than actual mutational changes within the TK gene locus.

In an in-vivo mouse micronucleus assay, groups of ICR mice (15/sex/dose) were administered single oral doses of 1,250, 2,500, or 5,000 mg/kg pelargonic acid. The bone marrow cells were harvested 24, 48, and 72 hours post-treatment. No significant increases in the frequency of micronucleated polychromatic erythrocytes (PCEs) were observed in either sex at any dose; thus, pelargonic acid was negative in the micronucleus assay.

A reverse gene mutation assay (Ames Test) concluded that pelargonic acid was not mutagenic under the conditions of the study.

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D. Aggregate Exposure

Pelargonic acid is a naturally-occurring fatty acid found in our food supply. Mycogen Corporation has estimated the potential worst case dietary exposure of pelargonic acid beyond existing natural background

levels after an application of Scythe Herbicide between grape vine rows. The commodity grape was selected because the use of Scythe Herbicide between grape vine rows is a representative and major use pattern intended for the product. In an effort to make a worst case scenario for residue calculations, Mycogen has suggested a 10 percent deposition on the crop, even though such a drift rate will be intentionally avoided by the grower because crop damage may result. Drift deposition would likely be less than 1 percent of applied spray volume.

The worst case human daily consumption level of pelargonic acid from treated grapes has been estimated to be 0.397 mg/kg/day. This exposure dose after applications of Scythe Herbicide must be compared to the highest dose level tested in the dietary range-finding toxicology study. In this study, a daily feeding dose of 1,834 mg/kg/day (20,000 ppm) did not produce any signs of toxicity or abnormalities for a period of 2 weeks.

Exposure to drinking water will be minimal. Scythe Herbicide will not be applied directly to water. The proposed label includes applications to dry ditches, dry canals, ditch banks, and for use above the water line or after draw-down of agricultural irrigation water and ditch systems, industrial ponds and disposal systems, and impounded water areas. Taking potential spray drift into consideration, the rapid degradation of pelargonic acid to naturally-occurring background levels in our environment will mitigate the exposure of residues to drinking water to insignificant amounts. In addition, the degradation of pelargonic acid will ensure that no contamination to groundwater will occur.

If residues of pelargonic acid do occur in food or in drinking water, information on the metabolism of fatty acids in the body confirms that residues of pelargonic acid would present minimal risk to humans. Fatty acids are digested in mammalian systems through normal metabolic pathways. While pelargonic acid is not as widespread in our diet as other fatty acids, the only difference is that most dietary fatty acids have even-numbered carbon chains and are ingested initially in the form of triglycerides. It is likely that pelargonic acid, when it is absorbed from the gastrointestinal tract into the blood, would be treated little differently from the free fatty acids released from adipose tissue.

Non-dietary exposure of pelargonic acid will be mitigated through the use of proper personal protective equipment. For non-occupational uses or exposure to sites not associated with food or drinking water, data on the natural occurrence and rapid microbiological degradation of pelargonic acid in the environment confirms that exposure will be minimal. EPA has waived all environmental fate data requirements for the current registration of Scythe Herbicide.

E. Cumulative Exposure

No cumulative exposure through other pesticides and substances with common mode of toxicity is expected. Pelargonic acid has a unique mode of action. Residues will not increase or sustain as a result of exposure to other materials. Pelargonic acid will degrade by microbial action to background levels over a period of 24 - 48 hours regardless of contact with substances either through pesticide tank mixing or exposure to other chemical residues in the environment. Normal use patterns will not lead to accumulation of pelargonic acid in the environment.

F. Safety Determination

Mycogen believes that the use of pelargonic acid as a naturally-occurring, lower toxicity, environmentally compatible material fits with EPA's objective to register reduced risk pesticides. The common dietary intake of the U.S. population includes low concentrations of naturally-occurring fatty acids, including pelargonic acid. The rapid environmental breakdown of pelargonic acid will significantly decrease any residues as a result of applications from Scythe Herbicide. Mycogen believes that under worst case exposure calculations, and based on established toxicology data, any increased levels of pelargonic acid will present no adverse effects to the consumer.

Mycogen believes that a determination of safety for infants and children can be made due to the insignificant exposure expected beyond naturally-occurring background levels, the fact that fatty acids are digested in mammalian systems through normal metabolic pathways, and the toxicology data base concludes that pelargonic acid is practically non-toxic when administered orally. The developmental toxicity screen study in rats produced a NOEL of 1,500 mg/kg/day (only dose tested), and no evidence of maternal or developmental toxicity was seen.

G. Existing Tolerances

Pelargonic acid is exempt under 40 CFR 180.1159 from the

requirement of a tolerance when used as a blossom thinning agent on apple and pear. Pelargonic acid has been added to the Food and Drug Administration's list of approved chemicals that may be safely used in washing or to assist in the lye peeling of fruits and vegetables in concentrations of up to 1 percent (21 CFR 173.315). The same use is cleared by the United States Department of Agriculture under the USDA List of Authorized Substances, 1990, 7 CFR 5.14, Fruit & Vegetable Washing Compounds. In addition, pelargonic acid is cleared by the Food and Drug Administration as a sanitizer solution to be used on food-contact articles [21 CFR 178.1010(b) (42)], or as a synthetic food flavoring agent and adjuvant (21 CFR 172.515).

II. Administrative Matters

Interested persons are invited to submit comments on this notice of filing. Comments must bear a notation indicating the document control number, [PF-685]. All written comments filed in response to this petition will be available in the Public Response and Program Resources Branch, at the address given above from 8:30 a.m. to 4 p.m., Monday through Friday, except legal holidays.

A record has been established for this notice of filing under docket number [PF-685] (including comments and data submitted electronically as described below). A public version of this record, including printed, paper versions of electronic comments, which does not include any information claimed as CBI, is available for inspection from 8:30 a.m. to 4 p.m., Monday through Friday, excluding legal holidays. The public record is located in Rm. 1132 of the Public Response and Program Resources Branch, Field Operations Division (7506C), Office of Pesticide Programs, Environmental Protection Agency, Crystal Mall #2, 1921 Jefferson Davis Hwy., Arlington, VA.

Electronic comments can be sent directly to EPA at:
opp-docket@epamail.epa.gov

Electronic comments must be submitted as an ASCII file avoiding the use of special characters and any form of encryption.

The official record for this notice of filing, as well as the public version, as described above will be kept in paper form. Accordingly, EPA will transfer all

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comments received electronically into printed, paper form as they are received and will place the paper copies in the official rulemaking record which will also include all comments submitted directly in writing. The official record is the paper record maintained at the address in ``ADDRESSES'' at the beginning of this document.

Authority: 21 U.S.C. 346a.

List of Subjects

Environmental protection, Agricultural commodities, Pesticides and pests, Reporting and recordkeeping requirements.

Dated: January 16, 1997.

Flora Chow,

Acting Director, Biopesticides and Pollution Prevention Division, Office of Pesticide Programs.

[FR Doc. 97-1753 Filed 1-23-97; 8:45 am]
BILLING CODE 6560-50-F

REFERENCE 2

(2)

CHAPTER 11 LIPIDS, LIPOPROTEINS, AND MEMBRANES

Lipids are water-insoluble organic biomolecules that can be extracted from cells and tissues by nonpolar solvents, e.g., chloroform, ether, or benzene. There are several different families or classes of lipids but all derive their distinctive properties from the hydrocarbon nature of a major portion of their structure. Lipids have several important biological functions, serving (1) as structural components of membranes, (2) as storage and transport forms of metabolic fuel, (3) as a protective coating on the surface of many organisms, and (4) as cell-surface components concerned in cell recognition, species specificity, and tissue immunity. Some substances classified among the lipids have intense biological activity; they include some of the vitamins and hormones.

Although lipids are a distinct class of biomolecules, we shall see that they often occur combined, either covalently or through weak bonds, with members of other classes of biomolecules to yield hybrid molecules such as *glycolipids*, which contain both carbohydrate and lipid groups, and *lipoproteins*, which contain both lipids and proteins. In such biomolecules the distinctive chemical and physical properties of their components are blended to fill specialized biological functions.

Classification of Lipids

Lipids have been classified in several different ways. The most satisfactory classification is based on their backbone structures (Table 11-1). The *complex lipids*, which characteristically contain fatty acids as components, include the *acylglycerols*, the *phosphoglycerides*, the *sphingolipids*, and the *waxes*, which differ in the backbone structures to which the fatty acids are covalently joined. They are also called *saponifiable lipids* since they yield soaps (salts of fatty acids) on alkaline hydrolysis. The other great group of lipids consists of the *simple lipids*, which do not contain fatty acids and hence are nonsaponifiable.

Let us first consider the structure and properties of fatty acids, characteristic components of all the complex lipids.

Table 11-1 Classification of lipids

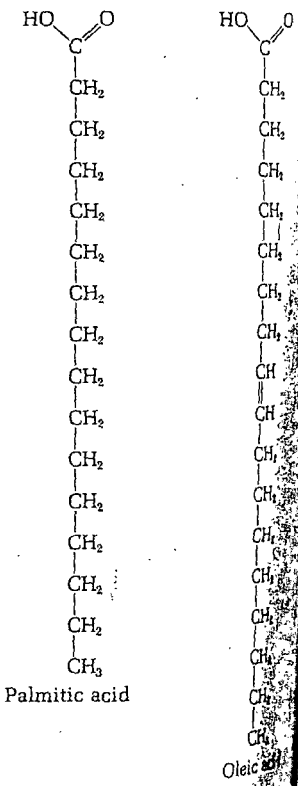
Lipid type	Backbone
Complex (saponifiable)	
Acylglycerols	Glycerol
Phosphoglycerides	Glycerol 3-phosphate
Sphingolipids	Sphingosine
Waxes	Nonpolar alcohols of high molecular weight
Simple (nonsaponifiable)	
Terpenes	
Steroids	
Prostaglandins	

Fatty Acids

Although fatty acids occur in very large amounts as building-block components of the saponifiable lipids, only traces occur in free (unesterified) form in cells and tissues. Well over 100 different kinds of fatty acids have been isolated from various lipids of animals, plants, and microorganisms. All possess a long hydrocarbon chain and a terminal carboxyl group (Figure 11-1). The hydrocarbon chain may be saturated, as in palmitic acid, or it may have one or more double bonds, as in oleic acid; a few fatty acids contain triple bonds. Fatty acids differ from each other primarily in chain length and in the number and position of their unsaturated bonds. They are often symbolized by a shorthand notation that designates the length of the carbon chain and the number, position, and configuration of the double bonds. Thus palmitic acid (16 carbons, saturated) is symbolized 16:0 and oleic acid [18 carbons and one double bond (cis) at carbons 9 and 10] is symbolized $18:1^{\Delta 9}$. It is understood that the double bonds are cis (see below) unless indicated otherwise. Table 11-2 gives the structures and symbols of some important saturated and unsaturated fatty acids and a few with unusual structures.

Some generalizations can be made on the different fatty acids of higher plants and animals. The most abundant have an even number of carbon atoms with chains between 14 and 22 carbon atoms long, but those with 16 or 18 carbons predominate. The most common among the saturated fatty acids are palmitic acid (C_{16}) and stearic acid (C_{18}) and among the unsaturated fatty acids oleic acid (C_{18}). Unsaturated fatty acids predominate over the saturated ones, particularly in higher plants and in animals living at low temperatures. Unsaturated fatty acids have lower melting points than saturated fatty acids of the same chain length (Table 11-2). In most monounsaturated (monoenoic) fatty acids of higher organisms there is a double bond between carbon atoms 9 and 10. In most polyunsaturated (polyenoic) fatty acids one double bond is between carbon atoms 9 and 10; the additional double bonds usually occur between the 9,10 double bond and the methyl-terminal end of the chain. In most types of polyunsaturated fatty acids the double bonds

Figure 11-1
Two common fatty acids.



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Terpenes	
Steroids	
Prostaglandins	

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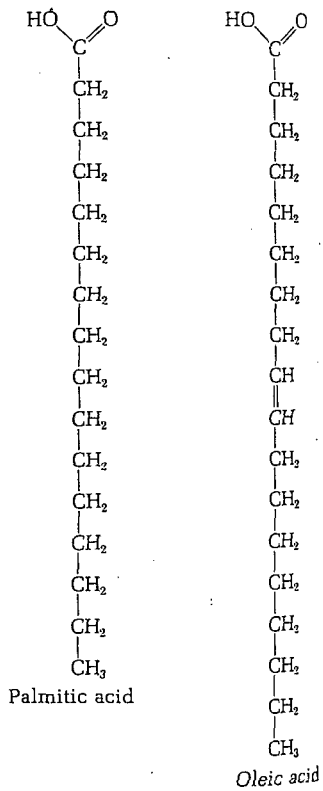


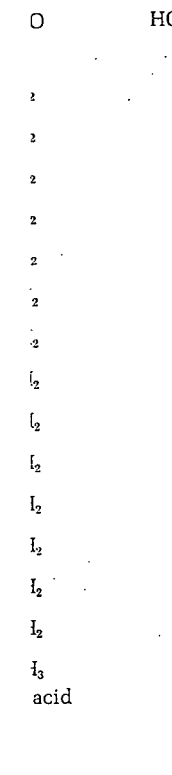
Palmitic acid

Oleic acid

Table 11-2 Some naturally occurring fatty acids

Symbol	Structure	Systematic name	Common name	m.p., °C
	Saturated fatty acids			
12:0	$CH_3(CH_2)_{10}COOH$	*n*-Dodecanoic	Lauric	44.2
14:0	$CH_3(CH_2)_{12}COOH$	*n*-Tetradecanoic	Myristic	53.9
16:0	$CH_3(CH_2)_{14}COOH$	*n*-Hexadecanoic	Palmitic	63.1
18:0	$CH_3(CH_2)_{16}COOH$	*n*-Octadecanoic	Stearic	69.6
20:0	$CH_3(CH_2)_{18}COOH$	*n*-Eicosanoic	Arachidic	76.5
24:0	$CH_3(CH_2)_{22}COOH$	*n*-Tetracosanoic	Lignoceric	86.0
	Unsaturated fatty acids			
16:1^{9}	$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$		Palmitoleic	−0.5
18:1^{9}	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$		Oleic	13.4
18:2^{9,12}	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$		Linoleic	−5
18:3^{9,12,15}	$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$		Linolenic	−11
20:4^{5,8,11,14}	$CH_3(CH_2)_4(CH{=}CHCH_2)_3CH{=}CH(CH_2)_3COOH$		Arachidonic	−49.5
	Some unusual fatty acids			
16:1^{9} trans	$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$ (*trans*)		*trans*-Hexadecenoic	
18:1^{9} trans	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$ (*trans*)		Elaidic	
	$CH_3(CH_2)_5HC\!-\!\!-\!CH(CH_2)_9COOH$ (CH_2 bridge)		Lactobacillic	
	$CH_3(CH_2)_7CH(CH_3)(CH_2)_8COOH$		Tuberculostearic	
	$CH_3(CH_2)_{21}CH(OH)COOH$		Cerebronic	

atty acids.



are separated by one methylene group, for example, —CH=CH—CH_2—CH=CH—; only in a few types of plant fatty acids are the double bonds in conjugation, that is, —CH=CH—CH=CH—. The double bonds of nearly all kinds of naturally occurring unsaturated fatty acids are in the cis geometrical configuration; only a very few are trans.

Bacteria contain fewer and simpler types of fatty acids than higher organisms, namely, C_{12} to C_{18} saturated acids, some of which have a branched methyl group (see Table 11-2), and also C_{16} and C_{18} monounsaturated acids. Fatty acids with more than one double bond have not been found in bacteria.

Fatty acids with an odd number of carbon atoms occur only in trace amounts in terrestrial animals but occur in significant amounts in many marine organisms.

Essential Fatty Acids

When weanling or immature rats are placed on a fat-free diet, they grow poorly, develop a scaly skin, lose hair, and ultimately die with many pathological signs. When linoleic acid is present in the diet, these conditions do not develop. Linolenic acid and arachidonic acid (Table 11-2) also prevent these symptoms. Saturated and monounsaturated fatty acids are inactive. It has been concluded that mammals can syn-

thesize saturated and monounsaturated fatty acids from other precursors but are unable to make linoleic and γ-linolenic acids. Fatty acids required in the diet of mammals are called *essential fatty acids*. The most abundant essential fatty acid in mammals is linoleic acid, which makes up from 10 to 20 percent of the total fatty acids of their triacylglycerols and phosphoglycerides. Linoleic and γ-linolenic acids cannot be synthesized by mammals but must be obtained from plant sources, in which they are very abundant. Linoleic acid is a necessary precursor in mammals for the biosynthesis of arachidonic acid, which is not found in plants.

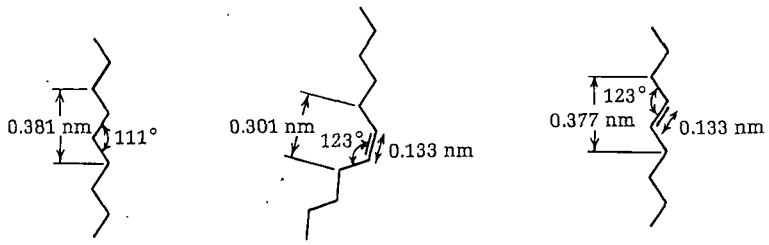
Although the specific functions of essential fatty acids in mammals were a mystery for many years, one function has been discovered. Essential fatty acids are necessary precursors in the biosynthesis of a group of fatty acid derivatives called *prostaglandins* (page 300), hormonelike compounds which in trace amounts have profound effects on a number of important physiological activities.

Physical and Chemical Properties of Fatty Acids

Saturated and unsaturated fatty acids have quite different conformations. In saturated fatty acids, the hydrocarbon tails are flexible and can exist in a very large number of conformations because each single bond in the backbone has complete freedom of rotation. The fully extended form shown in Figure 11-2, the minimum-energy form, is the most probable

Figure 11-2
Space-filling models of a saturated, a monounsaturated, and diunsaturated fatty acid (anionic forms).

Stearic acid Oleic acid Linoleic acid



Saturated chain

cis double bond (in most naturally occurring unsaturated fatty acids)

trans double bond (rare)

Figure 11-3
Geometry of double bonds in fatty acids.

conformation of saturated fatty acids. Unsaturated fatty acids, on the other hand, show one or more rigid kinks contributed by the nonrotating double bond(s). The cis configuration of the double bonds produces a bend of about 30° in the aliphatic chain (Figure 11-2), whereas the trans configuration more nearly resembles the extended form of saturated chains (Figure 11-3).

The cis forms of unsaturated fatty acids can be converted into trans forms by heating with certain catalysts. In this way oleic acid can be readily converted to its trans isomer *elaidic acid*, which has a much higher melting point (Table 11-2). Although elaidic acid is not a naturally occurring fatty acid, it is formed in appreciable amounts in the catalytic hydrogenation of liquid vegetable oils, a step in the manufacture of semisolid cooking fats and margarine. Elaidic acid has been found in the lipids of human tissues, presumably as a result of the consumption of such commercially hydrogenated products.

Unsaturated fatty acids undergo addition reactions at their double bonds. Quantitative titration with halogens, e.g., iodine or bromine, can yield information on the relative number of double bonds in a given sample of fatty acids or lipid.

Table 11-3 Fatty acid composition (percent) of lipids of mouse liver

	Phospho-glycerides	*Triacyl-glycerols*
Saturated		
Myristic	0	0
Palmitic	28	24
Stearic	20	4
Unsaturated		
Palmitoleic	4	6
Oleic	17	43
Linoleic	12	20
Linolenic	1	1
Arachidonic	18	2

Gas-Liquid Chromatography of Fatty Acids

Analysis of complex fatty acid mixtures obtained on hydrolysis of natural lipids was once an extremely difficult problem, but now precise and very sensitive analysis of fatty acid mixtures like that shown in Table 11-3 can be carried out by *gas-liquid chromatography*. In this procedure the fatty acids are first converted into a more volatile form, usually their methyl esters. An inert carrier gas such as nitrogen is used as the moving phase for partition chromatography of the vaporized mixture of methyl esters between the moving gas phase and a stationary liquid phase of a high-melting polyester or silicone polymer coated on particles of diatomaceous earth or on the inner surface of a long, heated capillary tube. The methyl esters of the various fatty acids partition themselves between the moving gas phase and the stationary liquid phase according to their individual gas-liquid partition coefficients. The separated methyl esters in

the gas phase leaving the column can be measured by a variety of extremely sensitive detectors. In one, the flame-ionization detector, the carrier gas stream containing the fatty acid esters is mixed with a stream of hydrogen and air and burned in a high-voltage electric field. The current generated by the flow of ionized fragments of the fatty acid in the flame is automatically recorded on a chart, which shows a series of separate peaks. Each peak corresponds to a separate fatty acid, and the area under the peak is proportional to the amount. Very complex mixtures of fatty acids can be sorted out in this fashion and quantitated; the amount of sample required for analysis is only a fraction of a milligram. Gas-liquid chromatography can also be used to analyze mixtures of sterols and hydrocarbons, as well as other compounds that are volatile at reasonable temperatures (up to 350°C) or can be converted chemically into volatile derivatives.

Triacylglycerols (Triglycerides)

Fatty acid esters of the alcohol glycerol (Figure 11-4) are called *acylglycerols* or *glycerides;* they are sometimes referred to as "neutral fats," a term that has become archaic. When all three hydroxyl groups of glycerol are esterified with fatty acids, the structure is called a *triacylglycerol* (Figures 11-4 and 11-5). (Although the name "triglyceride" has been traditionally used to designate these compounds, an international nomenclature commission has recommended that this chemically inaccurate term no longer be used.) Triacylglycerols are the most abundant family of lipids and the major components of depot or storage lipids in plant and animal cells. Triacylglycerols that are solid at room temperature are often referred to as "fats" and those which are liquid as "oils." *Diacylglycerols* (also called diglycerides) and *monoacylglycerols* (or monoglycerides) are also found in nature, but in much smaller amounts.

Triacylglycerols occur in many different types, according to the identity and position of the three fatty acid components esterified to glycerol. Those with a single kind of fatty acid in all three positions, called *simple* triacylglycerols, are named after the fatty acids they contain. Examples are *tristearoylglycerol*, *tripalmitoylglycerol*, and *trioleoylglycerol*; the trivial and more commonly used names are *tristearin*, *tripalmitin*, and *triolein*, respectively. *Mixed* triacylglycerols contain two or more different fatty acids. Triacylglycerols containing two different fatty acids A and B can exist in six different isomeric forms, BBA, AAB, ABA, ABB, BAA, and BAB, of which four (AAB, BAA, ABB, BBA) are stereoisomers (see below). The naming of mixed triacylglycerols can be illustrated by the example of 1-palmitoyldistearoylglycerol (trivial name, 1-palmitodistearin). Most natural fats are extremely complex mixtures of simple and mixed triacylglycerols.

Although there have been many attempts to discover the biological ground rules that determine the mode of distribution of different fatty acids in natural triacylglycerols, no simple, all-encompassing generalizations can yet be made.

Figure 11-4
Glycerol and mono-, di-, and triacylglycerols.

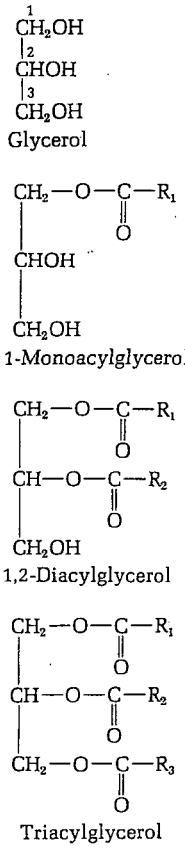


Figure 11-5
Space-filling model of 1-myristoyldipalmitoylglycerol, a mixed triacylglycerol.

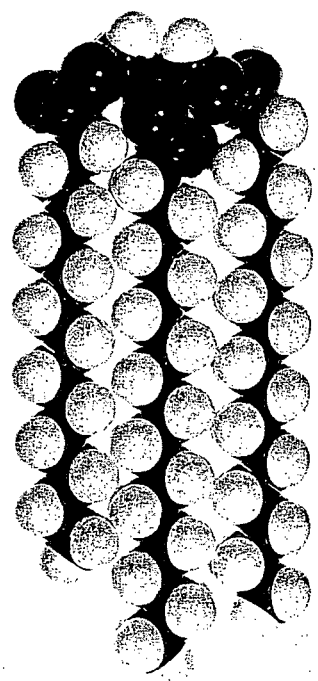


Table 11-4 shows the distribution of various types of triacylglycerols in the depot fat of the rat. The fatty acid composition of depot fat in part reflects the composition of the ingested lipids.

Table 11-4 Occurrence of simple and mixed triacylglycerols in depot fat of the rat (S = saturated; U = unsaturated)

Type	Symbol	Mol %
Simple	SSS	0.3
	UUU	61.8
Mixed	SSU	4.1
	SUS	1.6
	SUU	19.5
	USU	12.8

Properties of Triacylglycerols

The melting point of triacylglycerols is determined by their fatty acid components. In general, the melting point increases with the number and length of the saturated fatty acid components. For example, tripalmitin and tristearin are solids at body temperature, whereas triolein and trilinolein are liquids. All triacylglycerols are insoluble in water and do not tend by themselves to form highly dispersed micelles. However, diacylglycerols and monoacylglycerols have appreciable polarity because of their free hydroxyl groups and thus can form micelles (pages 43 and 300). Diacyl- and monoacylglycerols find wide use in the food industry in the production of more homogeneous and more easily processed foods; they are completely digestible and utilized biologically. Acylglycerols are soluble in ether, chloroform, benzene, and hot ethanol. Their specific gravity is lower than that of water.

Although glycerol itself is optically inactive, carbon atom 2 becomes asymmetric whenever the fatty acid substituents on carbon atoms 1 and 3 are different. Naturally occurring triacylglycerols with an asymmetric carbon atom are by convention named as if they were derived from L-glyceraldehyde.

Acylglycerols undergo hydrolysis when boiled with acids or bases or by the action of lipases, e.g., those present in pancreatic juice (Figure 11-6). Hydrolysis with alkali (page 279), called *saponification*, yields a mixture of soaps and glycerol.

Thin-Layer Chromatography

Triacylglycerols are separated and identified by the technique of thin-layer chromatography (Figure 11-7). A glass plate about 10 by 10 cm is covered with an aqueous slurry of an inert adsorbent material, such as silica gel or cellulose; the slurry also contains a binder such as plaster of paris. Sometimes silver nitrate is added, because Ag^+ forms weak bonds with unsaturated molecules, causing unsaturated acylglycerols to move more slowly than saturated ones. The

Figure 11-6
Hydrolysis of triacylglycerols.

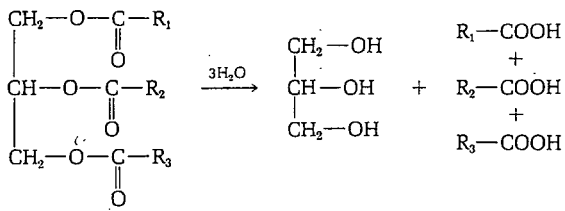
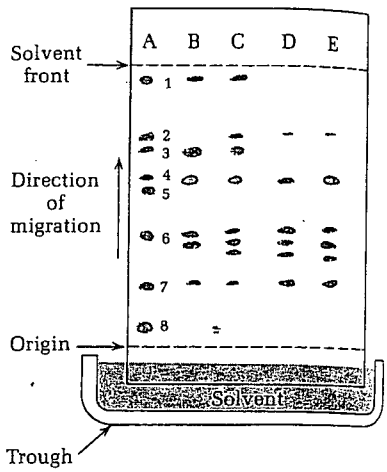


plate is air-dried and then baked to remove the remaining water, leaving a thin, uniform layer of firmly bound absorbent. The mixture to be analyzed is spotted at the bottom of the plate, and the lower edge of the plate is dipped into a pool of a suitable solvent in a closed chamber. The solvent rises by capillary action, as in paper chromatography, and the mixture of triacylglycerols is resolved into discrete spots. When the solvent front approaches the top, which takes only 20 to 30 min, the plate is dried and the positions of the separated components are located by spraying with a suitable indicator. The separated lipids can also be recovered by elution from patches of adsorbent scraped off the plate. This method can separate minute quantities of acylglycerols. Thin-layer chromatography is also useful for separating and identifying other types of lipids, as well as mixtures of amino acids, nucleotides, carbohydrates, and other cell components.

Figure 11-7
Thin-layer chromatography of acylglycerols on silica gel impregnated with $AgNO_3$. A = synthetic mixture, B = lard, C = cocoa butter, D = cottonseed oil, E = peanut oil. The spots are (1) tristearin, (2) 2-oleodistearin, (3) 1-oleodistearin, (4) 1-stearodiolein, (5) 1-linoleodistearin, (6) triolein, (7) trilinolein, and (8) monostearin.

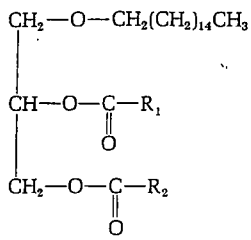


Alkyl Ether Acylglycerols

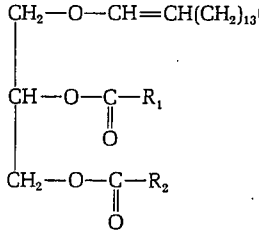
In addition to triacylglycerols, in which the three hydroxyl groups of glycerol are esterified with fatty acids, there is another family of closely related glycerol lipids, the *alkyl ether acylglycerols*, which are much less abundant than the triacylglycerols but occur widely. They contain fatty acids esterified to two of the hydroxyl groups of glycerol; the remaining hydroxyl group is joined in ether linkage with a long alkyl or alkenyl chain (Figure 11-8). These lipids are difficult to separate from the triacylglycerols; indeed, they escaped detection until the advent of refined chromatographic methods. Mild alkaline or enzymatic hydrolysis of the alkyl ether acylglycerols removes the fatty acyl groups to yield the *glyceryl ethers*, such as *chimyl* and *batyl* alcohols (Figure 11-8), hexadecyl and octadecyl ethers of glycerol, respectively.

Figure 11-8
Alkyl ether acylglycerols and glyceryl ethers.

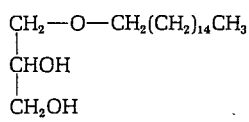
An alkyl ether diacylglycerol



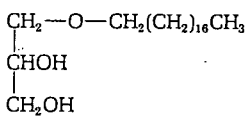
An α,β-alkenyl ether diacylglycerol



Two glyceryl ethers



Chimyl alcohol



Batyl alcohol

tography of acylglycerols
gnated with $AgNO_3$. A =
B = lard, C = cocoa but
l oil, E = peanut oil. The
rin, (2) 2-oleodistearin,
, (4) 1-stearodiolein, (5)
(6) triolein, (7) trilinolein
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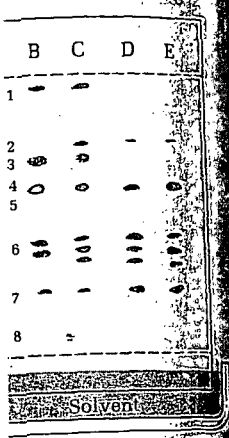
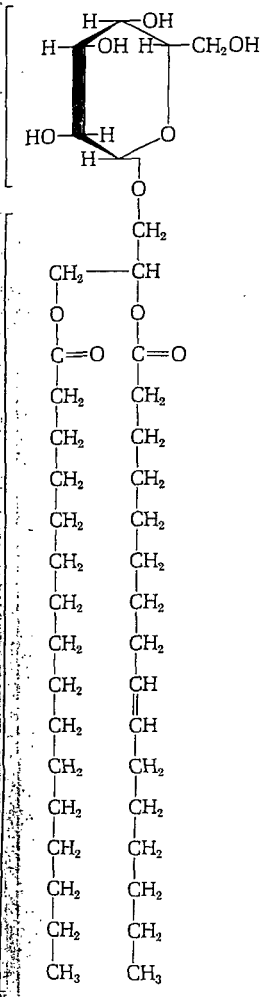


Figure 11-9
Galactosyldiacylglycerol.



Monogalactosyl diacylglycerol

Glycosylacylglycerols

Another family of acylglycerols includes the *glycosyldiacylglycerols*, which contain a sugar in glycosidic linkage with the unesterified 3-hydroxyl group of diacylglycerols. A common example is *galactosyldiacylglycerol* (Figure 11-9), found in higher plants and also in neural tissue of vertebrates. Similar glycolipids containing di- and trisaccharides are also known; a dimannosyldiacylglycerol has been isolated from bacteria.

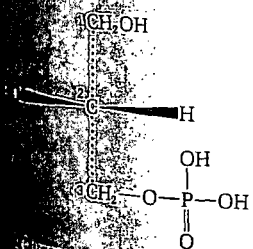
Phosphoglycerides

The second large class of complex lipids consists of the phosphoglycerides, also called *glycerol phosphatides*. They are characteristic major components of cell membranes; only very small amounts of phosphoglycerides occur elsewhere in cells. Phosphoglycerides are also loosely referred to as phospholipids or phosphatides, but it should be noted that not all phosphorus-containing lipids are phosphoglycerides; e.g., sphingomyelin (page 292) is a phospholipid because it contains phosphorus, but it is better classified as a sphingolipid because of the nature of the backbone structure to which the fatty acid is attached.

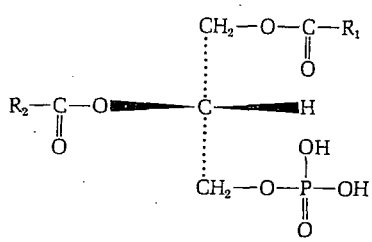
In phosphoglycerides one of the primary hydroxyl groups of glycerol is esterified to phosphoric acid; the other hydroxyl groups are esterified to fatty acids. The parent compound of the series is thus the phosphoric ester of glycerol. This compound has an asymmetric carbon atom and can be designated as either D-glycerol 1-phosphate or L-glycerol 3-phosphate. Because of this ambiguity, the stereochemistry of glycerol derivatives is based on the *stereospecific numbering* (*sn*) of the carbon atoms, as shown in Figure 11-10. The isomer of glycerol phosphate found in natural phosphoglycerides is called *sn*-glycerol 3-phosphate; it belongs to the L-stereochemical series. In addition to the two fatty acid residues esterified to the hydroxyl groups at carbon atoms 1 and 2, phosphoglycerides contain a polar head group, namely, an alcohol designated X—OH, whose hydroxyl group is esterified to the phosphoric acid.

Because phosphoglycerides possess a polar head in addition to their nonpolar hydrocarbon tails (Figure 11-11), they are called *amphipathic* (page 43) or *polar* lipids. The different types of phosphoglycerides differ in the size, shape,

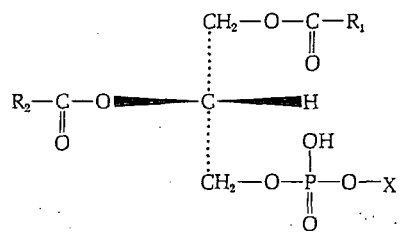
...ure 11-10
...chemical configuration of phospho-
...rides.



...ycerol 3-phosphoric acid
...erol 3-phosphoric acid)



L-Phosphatidic acid
(3-*sn*-phosphatidic acid)



General structure of phosphoglycerides. The moiety X is contributed by an alcohol.

and electric charge of their polar head groups (Table 11-5 and Figures 11-11 and 11-12). Each type of phosphoglyceride can exist in many different chemical species differing in their fatty acid substituents. Usually there is one saturated and one unsaturated fatty acid, the latter in the 2 position of glycerol.

The parent compound of the phosphoglycerides is *phosphatidic acid* (Figure 11-10), which contains no polar alcohol head group. It occurs in only very small amounts in cells, but it is an important intermediate in the biosynthesis of the phosphoglycerides. The most abundant phosphoglycerides in higher plants and animals are *phosphatidylethanolamine* and *phosphatidylcholine* (Table 11-5 and Figure 11-12), which contain as head groups the amino alcohols *ethanolamine* and *choline*, respectively. (The new names recommended for these phosphoglycerides are *ethanolamine phosphoglyceride* and *choline phosphoglyceride*, but they have not yet gained wide use. The old trivial names are cephalin and lecithin, respectively.) These two phosphoglycerides are major components of most animal cell membranes.

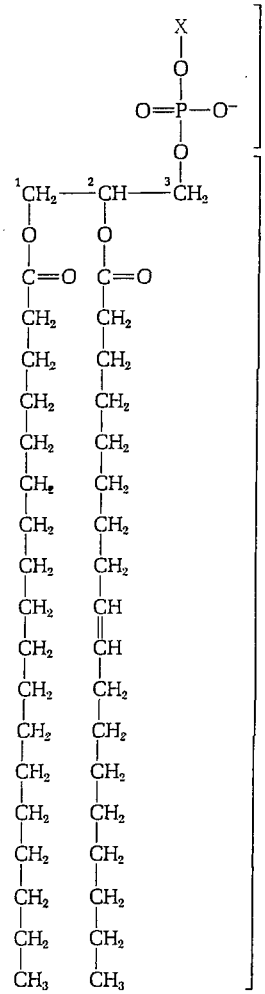
In *phosphatidylserine*, the hydroxyl group of the amino acid L-serine is esterified to the phosphoric acid. In *phosphatidylinositol*, the head group is the six-carbon cyclic sugar alcohol inositol. In *phosphatidylglycerol*, the head group is a molecule of glycerol. Phosphatidylglycerol is often found in bacterial membranes as an amino acid derivative, particularly of L-lysine, which is esterified at the 3′ position of the glycerol head group. This type of amino acid–containing lipid is called a *lipoamino acid* or, more accurately, an *O-aminoacylphosphatidylglycerol* (see Table 11-5).

Closely related to phosphatidylglycerol is the more complex lipid *cardiolipin*, also called *diphosphatidylglycerol*, which consists of a molecule of phosphatidylglycerol in which the 3′-hydroxyl group of the second glycerol moiety is esterified to the phosphate group of a molecule of phosphatidic acid (Figure 11-12 and Table 11-5). The backbone of cardiolipin thus consists of three molecules of glycerol joined by two phosphodiester bridges; the two hydroxyl groups of both external glycerol molecules are esterified with fatty acids. Phosphatidylglycerol, *O*-aminoacylphosphatidylglycerol, and cardiolipin are therefore structurally related. They are characteristically abundant in the cell membranes of bacteria. Cardiolipin is also present in large amounts in the inner membrane of mitochondria; it was first isolated from heart muscle, in which mitochondria are abundant.

The polar head groups of phosphatides may also be contributed by a sugar molecule. Phosphatidyl sugars have been found in plants and microorganisms. They are not to be confused with other types of glycolipids containing no phosphoric acid.

Plasmalogens differ from all the other phosphoglycerides described above. One of the two hydrocarbon tails is contributed by a long-chain fatty acid esterified to the 2 position of the glycerol, but the other is a long aliphatic chain in cis

Figure 11-11
General structure of phosphoglycerides in a form emphasizing their amphipathic nature. Usually the fatty acid in the 2 position is unsaturated.



of phosphoglycerides in
their amphipathic nature
acid in the 2 position is

Polar head

Nonpolar tails

Table 11-5 Polar head groups of the phosphoglycerides

The head alcohols are shown in color. The open bonds on the phosphoric residues are to position 3 of 1,2-diacylglycerol.

Phosphatidylethanolamine

Phosphatidylcholine

Phosphatidylserine

Phosphatidylinositol

Phosphatidylglycerol

3′-O-Lysylphosphatidylglycerol

Cardiolipin (diphosphatidylglycerol)

Figure 11-12
Space-filling models of three phosphoglycerides, showing location of the charged groups.

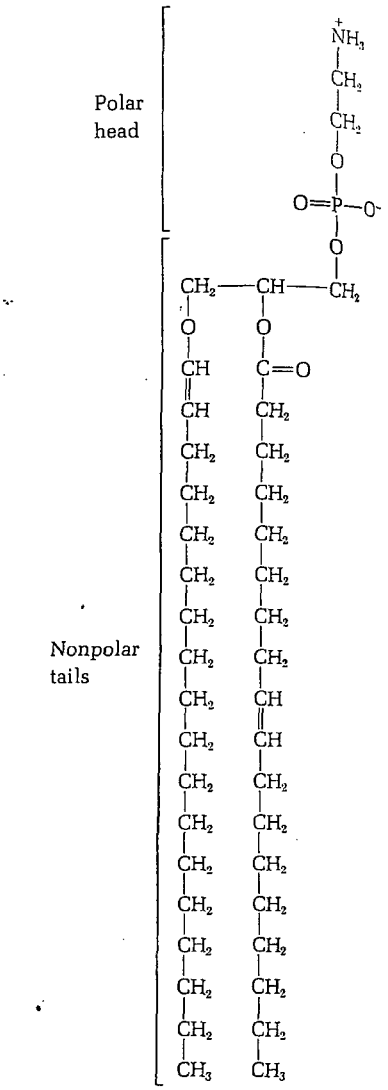
Phosphatidyl-ethanolamine

Phosphatidyl-choline

Cardiolipin

α,β-unsaturated ether linkage at the 1 position (Figure 11-13). Thus plasmalogens are phosphoglyceride analogs of the alkyl ether acylglycerols described above (page 286); ethanolamine is the most common polar head group. They are especially abundant in the membranes of muscle and nerve cells.

Figure 11-13
A plasmalogen



Properties of Phosphoglycerides

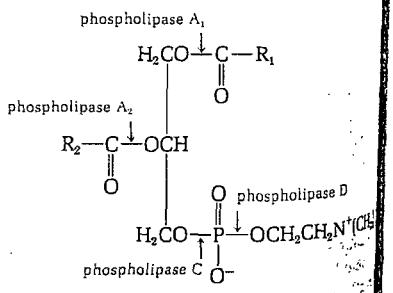
Pure phosphoglycerides are white waxy solids. On exposure to air they darken and undergo complex chemical changes because of the tendency of their polyunsaturated fatty acid components to be peroxidized by atmospheric oxygen, which results in polymerization. Phosphoglycerides are soluble in most nonpolar solvents containing some water and are best extracted from cells and tissues with chloroform-methanol mixtures. They are not readily soluble in anhydrous acetone. When phosphoglycerides are placed in water, they appear to dissolve, but only very minute amounts go into true solution; most of the "dissolved" lipid is in the form of micelles (see below).

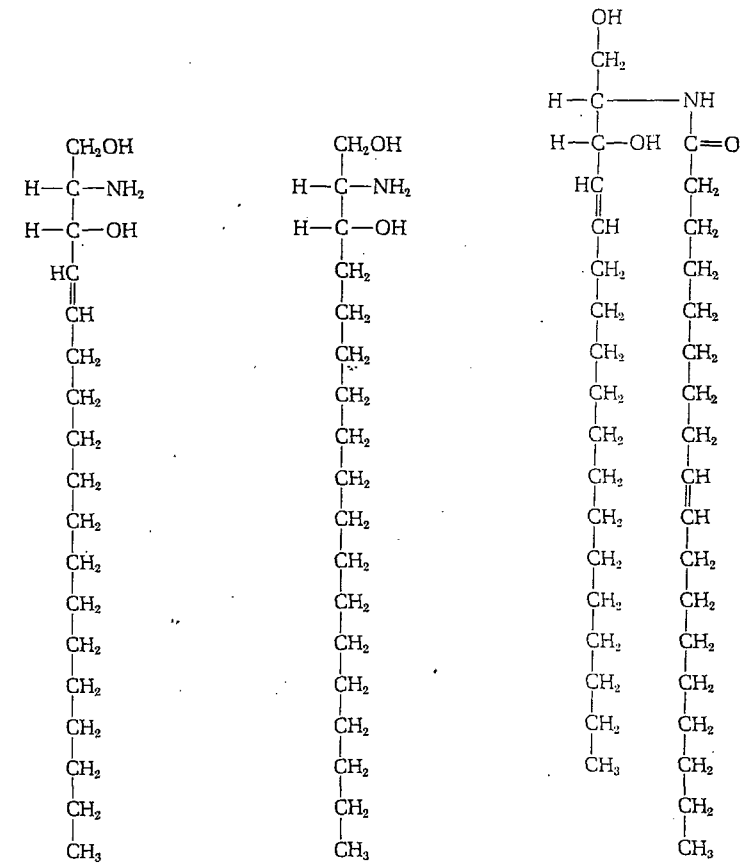
All phosphoglycerides have a negative charge at the phosphate group at pH 7; the pK' of this group is in the range of 1 to 2. The head groups of phosphatidylinositol, phosphatidylglycerol, and the phosphatidyl sugars have no electric charge, but they are quite polar because of their high content of hydroxyl groups. The head groups of phosphatidylethanolamine and phosphatidylcholine have a positive charge at pH 7; thus at this pH these two phosphoglycerides are dipolar zwitterions with no net electric charge. The head group of phosphatidylserine contains an α-amino group ($pK' = 10$) and a carboxyl group ($pK' = 3$); the phosphatidylserine molecule thus contains two negative charges and one positive charge at pH 7.0, giving it a net negative charge. *O*-Lysylphosphatidylglycerol, on the other hand, with two positive charges and one negative charge at pH 7.0 has a net positive charge. These variations in the size, shape, polarity, and electric charge of the polar heads (Table 11-5 and Figure 11-12) presumably play a significant role in the structure of various types of cell membranes (page 302).

Mild alkaline hydrolysis of phosphoglycerides yields the fatty acids as soaps but leaves the glycerol–phosphoric acid–alcohol portion of the molecule intact. For example, hydrolysis of phosphatidylcholine under these conditions yields glycerol 3-phosphorylcholine. Hydrolysis of phosphoglycerides with strong alkali causes hydrolytic cleavage not only of the fatty acids but also the head alcohol; since the linkage between phosphoric acid and glycerol is relatively stable to alkaline hydrolysis, the other product is glycerol phosphate, which can be cleaved by acid hydrolysis.

Phosphoglycerides can also be hydrolyzed by specific phospholipases, which have become important tools in the determination of phosphoglyceride structure (Figure 11-14). Phospholipase A_1 specifically removes the fatty acid from the 1 position and phospholipase A_2 from the 2 position. Removal of one fatty acid molecule from a phosphoglyceride

Figure 11-14
Sites of action of phospholipases on phosphatidylcholine.





yields a *lysophosphoglyceride*, e.g., *lysophosphatidylethanolamine*. Lysophosphoglycerides are intermediates in phosphoglyceride metabolism but are found in cells or tissues in only very small amounts; in high concentrations they are toxic and injurious to membranes. Phospholipase B, a mixture of phospholipases A_1 and A_2, can bring about successive removal of the two fatty acids of phosphoglycerides. Phospholipase C hydrolyzes the bond between phosphoric acid and glycerol, while phospholipase D removes the polar head group to leave a phosphatidic acid.

Phosphoglycerides, like other complex lipids described below, are readily separated and identified by thin-layer chromatography or by chromatography on silicic acid columns.

Sphingolipids

Sphingolipids, complex lipids containing as their backbone *sphingosine* or a related base (Figure 11-15), are important membrane components in both plant and animal cells. They are present in especially large amounts in brain and nerve tissue. Only trace amounts of sphingolipids are found in depot fats. All sphingolipids contain three characteristic

building-block components: one molecule of a fatty acid, one molecule of sphingosine or one of its derivatives, and a polar head group, which in some sphingolipids is very large and complex.

Sphingosine (Figure 11-15) is one of 30 or more different long-chain amino alcohols found in sphingolipids of various species. In mammals *sphingosine* (4-sphingenine) and *dihydrosphingosine* (sphinganine) are the major bases of sphingolipids, in higher plants and yeast *phytosphingosine* (4-hydroxysphinganine) is the major base, and in marine invertebrates doubly unsaturated bases such as *4,8-sphingadiene* are common. The sphingosine base is connected at its amino group by an amide linkage to a long saturated or monounsaturated fatty acid of 18 to 26 carbon atoms. The resulting compound, which has two nonpolar tails and is called a *ceramide* (Figure 11-15), is the characteristic parent structure of all sphingolipids. Different polar head groups are attached to the hydroxyl group at the 1 position of the sphingosine base.

Sphingomyelins

The most abundant sphingolipids in the tissues of higher animals are *sphingomyelins*, which contain phosphorylethanolamine or phosphorylcholine as their polar head groups, esterified to the 1-hydroxyl group of ceramide (Figure 11-16). Sphingomyelins have physical properties very similar to those of phosphatidylethanolamine and phosphatidylcholine; they are zwitterions at pH 7.0.

Neutral Glycosphingolipids

A second class of sphingolipids contains one or more neutral sugar residues as their polar head groups and thus has no electric charge; they are called neutral glycosphingolipids. The simplest of these are the *cerebrosides*, which contain as their polar head group a monosaccharide bound in β glycosidic linkage to the hydroxyl group of ceramide (Figure 11-17). The cerebrosides of the brain and nervous system contain D-galactose and are therefore called *galactocerebrosides*. Cerebrosides are also present in much smaller amounts in nonneural tissues of animals, where, because they usually contain D-glucose instead of D-galactose, they are called *glucocerebrosides*. Sulfate esters of galactocerebrosides (at the 3 position of the D-galactose) are also present in brain tissue; they are called *sulfatides*. Cerebrosides and sulfatides usually contain fatty acids with 22 to 26 carbon atoms. A common fatty acid component of cerebrosides is *cerebronic acid* (Table 11-2), which has a D-hydroxyl group at carbon atom 2. When the fatty acid is cleaved from a cerebroside by alkaline hydrolysis, the remaining glycosylsphingosine compound is called a *psychosine*.

Neutral glycosphingolipids with disaccharides as their polar head groups are called *dihexosides*. Also known are *trihexosides* and *tetrahexosides* (Table 11-6), containing tri-

Figure 11-16
Structure of a representative sphingomyelin.

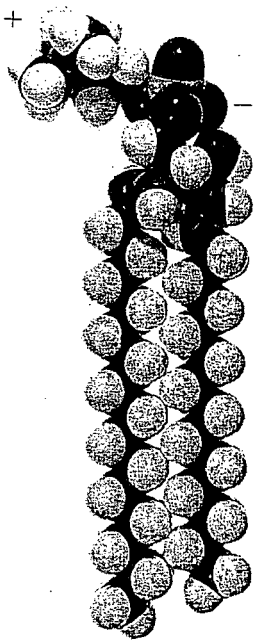
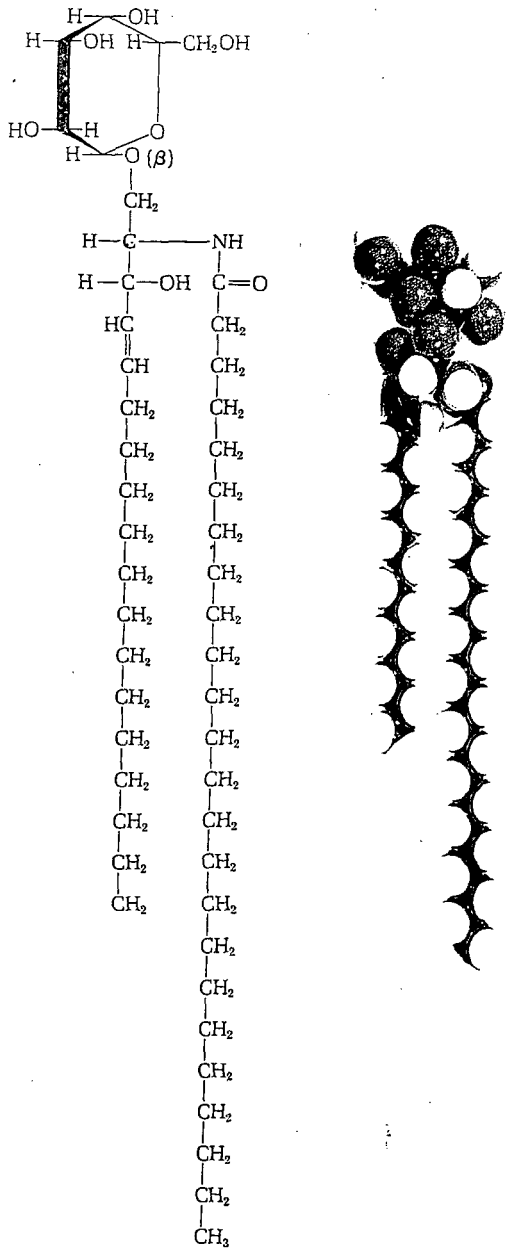


Figure 11-17
A galactocerebroside containing lignoceric acid (C_{24}) as its fatty acid component.



saccharide and tetrasaccharide head groups, respectively. The monosaccharide units found in these glycosphingolipids include D-glucose, D-galactose, *N*-acetyl-D-glucosamine, and *N*-acetyl-D-galactosamine. The neutral glycosphingolipids are important cell-surface components in animal tissues. Their nonpolar tails presumably penetrate into the lipid bilayer structure of cell membranes (see page 305), whereas the polar heads protrude outward from the surface. Some of the neutral glycosphingolipids are found on the surface of red blood cells and give them blood-group specificity; they

Table 11-6 Major classes of neutral glycosphingolipids. The symbols are Glc, D-glucose; Gal, D-galactose; GalNAc, *N*-acetyl-D-galactosamine.

Glucosylceramides	
Monohexoside (glucocerebroside)	Glc $1\xrightarrow{\beta}$ceramide
Dihexoside	Gal $1\xrightarrow{\beta}4$ Glc $1\xrightarrow{\beta}$ceramide
Trihexoside	Gal $1\xrightarrow{\alpha}4$ Gal $1\xrightarrow{\beta}4$ Glc $1\xrightarrow{\beta}$ceramide
Tetrahexoside	GalNAc $1\xrightarrow{\beta}3$ Gal $1\xrightarrow{\alpha}4$ Gal $1\xrightarrow{\beta}4$ Glc $1\xrightarrow{\beta}$ceramide
Galactosylceramides	
Galactocerebroside	Gal $1\xrightarrow{\beta}$ceramide
Dihexoside	Gal $1\xrightarrow{\beta}4$ Gal $1\xrightarrow{\beta}$ceramide

are responsible, in part, for the need to match donor and recipient blood for compatibility.

The neutral glycosphingolipids are classified on the basis of the identity of the sugar attached to the ceramide unit, the sequence of the sugars, and the length of the oligosaccharide chains. Examples of some glycosphingolipids are shown in Table 11-6.

Acidic Glycosphingolipids (Gangliosides)

The third and most complex group of glycosphingolipids are the *gangliosides;* they contain in their oligosaccharide head groups one or more residues of a sialic acid (page 650), which gives the polar head of the gangliosides a net negative charge at pH 7.0 (Table 11-7). The sialic acid usually found in human gangliosides is *N*-acetylneuraminic acid (page 260). Gangliosides are most abundant in the gray matter of the brain, where they constitute 6 percent of the total lipids, but small amounts are also found in nonneural tissues. Over 20 different types of gangliosides have been identified, differing in the number and relative positions of the hexose and sialic acid residues, which form the basis of their classification (Table 11-7). Nearly all the known gangliosides have a glucose residue in glycosidic linkage with ceramide; residues of D-galactose and *N*-acetyl-D-galactosamine are also present.

Function of Glycosphingolipids

Much attention is now focused on the biochemistry of the glycosphingolipids. Although they are only minor constituents of membranes, they appear to be extremely important in a number of specialized functions. Because gangliosides are especially abundant in nerve endings, it has been suggested that they function in the transmission of nerve impulses across synapses. They are also believed to be present at receptor sites for acetylcholine and other neurotransmitter substances. Some of the cell-surface glycosphingolipids are concerned not only in blood-group specificity but also in organ and tissue specificity. These complex lipids are also involved in tissue immunity and in cell-cell recognition sites fundamental to the development and structure of tissues. Cancer cells, for example, have characteristic glycosphingolipids different from those in normal cells. Ganglioside G_{M2}

Table 11-7 Structures of some gangliosides†

	Symbol
NANA 2→3 Gal 1$\xrightarrow{\beta}$4 Glc 1$\xrightarrow{\beta}$ceramide	G_{M3}
GalNAc 1$\xrightarrow{\beta}$4 Gal 1$\xrightarrow{\beta}$4 Glc 1$\xrightarrow{\beta}$ceramide 3 ↑ 2 NANA	G_{M2}
3 GalNAc 1$\xrightarrow{\beta}$4 Gal 1$\xrightarrow{\beta}$4 Glc 1$\xrightarrow{\beta}$ceramide ↑β 3 1 Gal ↑ 2 NANA	G_{M1}
3 GalNAc 1→4 Gal 1$\xrightarrow{\beta}$4 Glc 1→ceramide ↑β 3 1 Gl ↑ 3 2 NANA ↑ NANA 2	G_{D1}
3 GalNAc 1$\xrightarrow{\beta}$4 Gal 1$\xrightarrow{\beta}$4 Glc 1$\xrightarrow{\beta}$ceramide ↑β 3 1 Gal ↑ 3 2NANA 8 ↑ ↑ NANA 2 2 NANA	G_{T1}

† Glc = D-glucose, Gal = D-galactose, GalNAc = *N*-acetyl-D-galactosamine, NANA = *N*-acetylneuramic acid (sialic acid). In this nomenclature of gangliosides, devised by L. Svennerholm, the subscript letters indicate the number of sialic acid groups (M = monosialo, D = disialo, and T = trisialo). The numeral in the subscript is $5 - n$, where n is the number of neutral sugar residues. The sialic acid residues are in color.

accumulates in the brain in Tay-Sachs disease, due to genetic lack of the enzyme required for its degradation (page 678). Several other genetic deficiency diseases result in abnormal accumulation of different glycosphingolipids (page 678).

Waxes

Waxes are water-insoluble, solid esters of higher fatty acids with long-chain monohydroxylic fatty alcohols or with sterols (see below). They are soft and pliable when warm but hard when cold. Waxes are found as protective coatings on skin, fur, and feathers, on leaves and fruits of higher plants, and on the exoskeleton of many insects. The major components of beeswax are palmitic acid esters of long-chain fatty alcohols with 26 to 34 carbon atoms. Lanolin, or wool fat, is a mixture of fatty acid esters of the sterols lanosterol and agnosterol (see below).

Simple (Nonsaponifiable) Lipids

The lipids discussed up to this point contain fatty acids as building blocks, which can be released on alkaline hydrolysis. The simple lipids contain no fatty acids. They occur in smaller amounts in cells and tissues than the complex lipids, but they include many substances having profound biological activity—vitamins, hormones, and other highly specialized fat-soluble biomolecules.

There are two major classes of nonsaponifiable lipids, the terpenes and the steroids. Although it is convenient to consider them as two distinct classes, they are closely related structurally, since both ultimately derive from five-carbon building blocks.

Terpenes

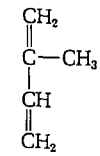
Terpenes are constructed of multiples of the five-carbon hydrocarbon isoprene (2-methyl-1,3-butadiene) (Figure 11-18). Terpenes containing two isoprene units are called monoterpenes, those containing three isoprene units are called sesquiterpenes, and those containing four, six, and eight units are called diterpenes, triterpenes, and tetraterpenes, respectively. Terpenes may be either linear or cyclic molecules; some terpenes contain structures of both types. The successive isoprene units of terpenes are usually linked in a head-to-tail arrangement, particularly in the linear segments, but sometimes the isoprene units are in tail-to-tail arrangement. The double bonds in the linear segments of most terpenes are in the stable trans configuration, but in some, particularly vitamin A and its precursor β-carotene (below), one or more of the double bonds are cis.

Of the very large number of terpenes identified in plants, many have characteristic odors or flavors and are major components of essential oils derived from such plants. Thus the monoterpenes geraniol, limonene, menthol, pinene, camphor, and carvone are major components of oil of geranium, lemon oil, mint oil, turpentine, camphor oil, and caraway oil, respectively. Farnesol is an example of a sesquiterpene. The diterpenes include phytol, a linear terpenoid alcohol, which is a component of the photosynthetic pigment chlorophyll (page 595). The triterpenes include squalene, an important precursor in the biosynthesis of cholesterol. Other higher terpenes include the carotenoids, a class of tetraterpene hydrocarbons and their oxygen-containing derivatives in which the head-to-tail arrangement of the isoprene units is characteristically reversed at the center of the molecule (Figure 11-19). An important carotenoid is β-carotene, the hydrocarbon precursor of vitamin A. Natural rubber and gutta-percha are polyterpenes; they consist of long hydrocarbon chains containing hundreds of isoprene units in regular linear order.

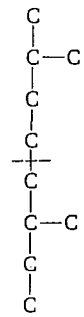
Among the most important terpenes are three members of the group of fat-soluble vitamins, namely, vitamins A, E, and K. Although these substances, which are required in trace amounts in the diet of mammals, may be classified among the lipids, their biological functions are so distinctive that their structure and function will be considered separately in Chapter 13, page 351.

Another important class of terpenes is represented by the polyprenols, long-chain linear polyisoprenoid compounds with a terminal primary alcohol group. The most important of these is undecaprenyl alcohol, also called bactoprenol, which contains 11 isoprene units and thus has 55 carbon atoms (Figure 11-19). Dolichol is the corresponding analog in

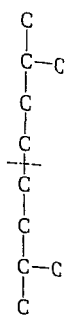
Figure 11-18
Isoprene units in the structure of some simple terpenes.



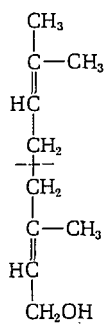
Isoprene



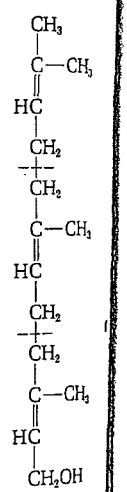
Head-to-tail or regular arrangement of isoprene units



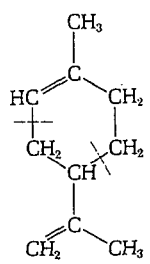
Tail-to-tail or irregular arrangement of isoprene units



Geraniol, a linear monoterpene



Farnesol, a linear sesquiterpene



Limonene, a cyclic monoterpene

the structure of some sim

its

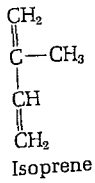
l3

H3

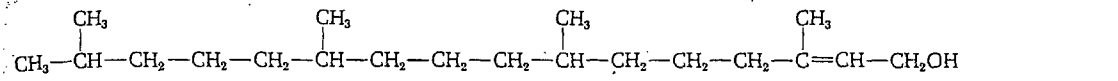
H

l, a
terpene

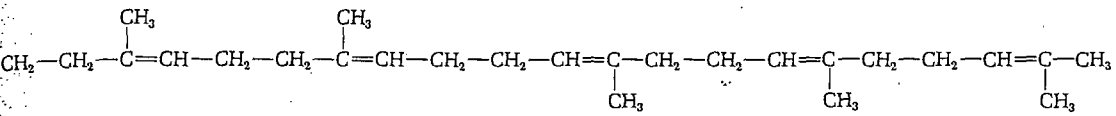
Figure 11-19
Some higher terpenes. Terpene structures are often shown in shorthand notation.



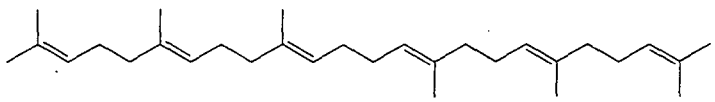
Isoprene



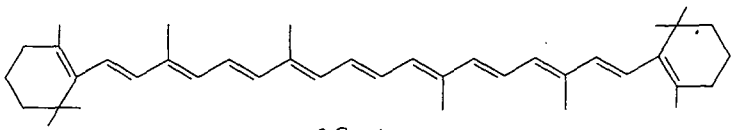
Phytol



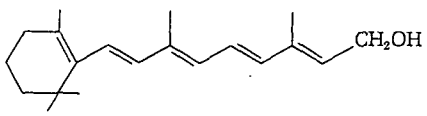
Squalene



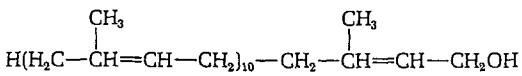
Squalene (shorthand form)



β-Carotene



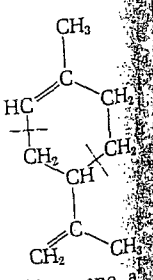
Vitamin A_1 (retinol)



Bactoprenol

Tail-to-tail or irregular arrangement of isoprene u

Farnesol, linear sesquiterpene



Limonene, a cyclic monoterpene

animal tissues; it contains 19 isoprene units (95 carbon atoms). These polyprenols, in the form of their phosphate esters, *undecaprenyl phosphate* and *dolichyl phosphate*, respectively, have a coenzymelike function in the enzymatic transfer of sugar groups from the cytoplasm to the outer surface of the cell during the synthesis of cell-surface and cell-wall lipopolysaccharides, peptidoglycans, teichoic acids, and glycoproteins (pages 268, 273, and 649). In this process the long, nonpolar hydrocarbon chain of the polyprenols is believed to be anchored within the nonpolar core of the membrane (page 302), whereas the polar end of the molecule serves as an arm for the transfer of the covalently bound sugar groups across the membrane.

Still another class of terpenoid compounds functioning as coenzymes is the *ubiquinone* or *coenzyme Q* family of compounds, which function as hydrogen carriers for biological

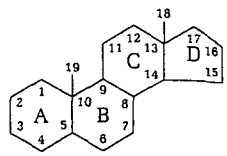
oxidations in the mitochondria (page 493). They contain a substituted quinone ring, which can be reduced and then reoxidized, and a long isoprenoid side chain, whose length differs with the organism. Analogous compounds, called *plastoquinones*, are found in chloroplasts, where they function in photosynthesis (page 607).

Steroids

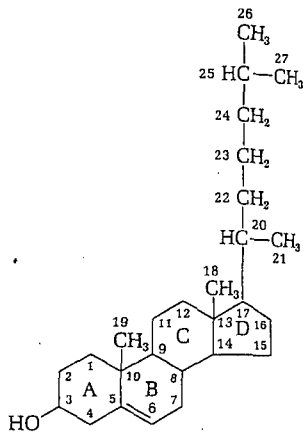
Steroids are derivatives of the saturated tetracylic hydrocarbon *perhydrocyclopentanophenanthrene* (Figure 11-20). A great many different steroids, each with a distinctive function or activity, have been isolated from natural sources. Steroids differ in the number and position of double bonds, in the type, location, and number of substituent functional groups, in the configuration (α or β) of the bonds between the substituent groups and the nucleus, and in the configuration of the rings in relation to each other, since the parent hydrocarbon has six centers of asymmetry. The main points of substitution are carbon 3 of ring A, carbon 11 of ring C, and carbon 17 of ring D. All steroids originate from the linear triterpene *squalene* (Figure 11-19), which cyclizes readily (page 683). The first important steroid product of this cyclization is *lanosterol*, which in animal tissues is the precursor of *cholesterol*, the most abundant steroid in animal tissues. Cholesterol and lanosterol are members of a large subgroup of steroids called the *sterols*. They are steroid alcohols containing a hydroxyl group at carbon 3 of ring A and a branched aliphatic chain of eight or more carbon atoms at carbon 17. They occur either as free alcohols or as long-chain fatty acid esters of the hydroxyl group at carbon 3; all are solids at room temperature. *Cholesterol* melts at 150°C and is insoluble in water but readily extracted from tissues with chloroform, ether, benzene, or hot alcohol. Cholesterol occurs in the plasma membranes of many animal cells and in the lipoproteins of blood plasma. *Lanosterol* (Figure 11-20) was first found in the waxy coating of wool in esterified form before it was established as an important intermediate in the biosynthesis of cholesterol in animal tissues (page 683). Cholesterol occurs only rarely in higher plants, which contain other types of sterols known collectively as *phytosterols*. Among these are *stigmasterol* and *sitosterol*. Fungi and yeasts contain still other types of sterols, the *mycosterols*. Among these is *ergosterol*, which is converted to vitamin D on irradiation by sunlight. Sterols are not present in bacteria.

Cholesterol is the precursor of many other steroids in animal tissues, including the *bile acids*, detergentlike compounds that aid in emulsification and absorption of lipids in the intestine; the *androgens*, or male sex hormones; the *estrogens*, or female sex hormones; the progestational hormone *progesterone*; and the *adrenocortical hormones* (Figure 11-21). The biological activity of some of the steroid hormones will be discussed in Chapter 29, pages 823 and 824. Among the most important steroids are a group of compounds having vitamin D activity; their structure and function will be discussed in Chapter 13 (page 355).

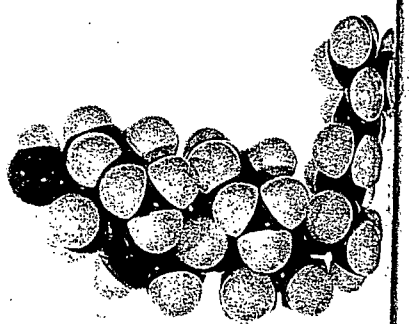
Figure 11-20
The structures of the steroid nucleus, cholesterol, and lanosterol. The ring designations and numbering of the carbon atoms of steroids are also shown.



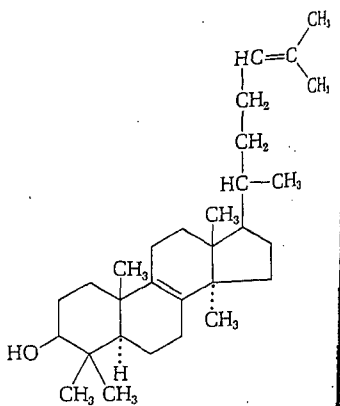
Perhydrocyclopentanophenanthrene nucleus



Cholesterol

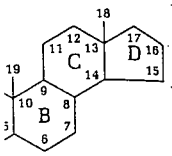


Space-filling model of cholesterol

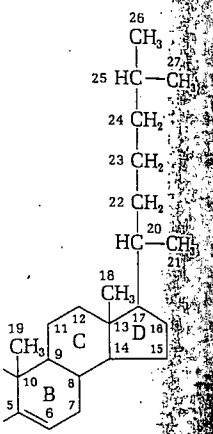


Lanosterol

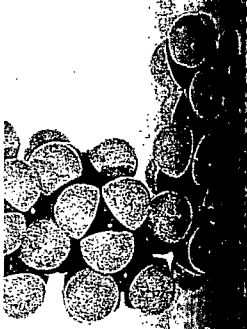
the steroid nucleus,
lanosterol. The ring de-
mbering of the carbon
are also shown.



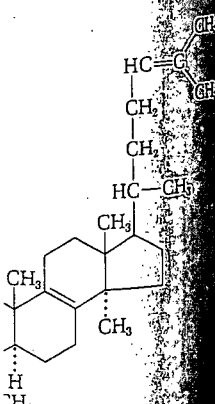
clopentanophenanthrene
nucleus



Cholesterol



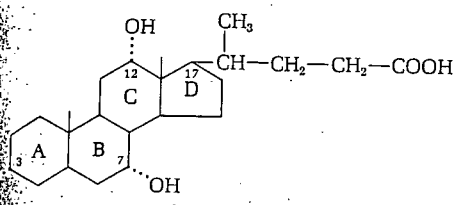
ng model of cholesterol



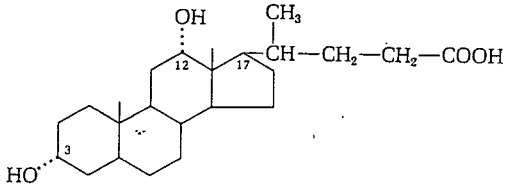
Lanosterol

Figure 11-21
Some important steroids. The two bile acids usually occur as amides of glycine and taurine.

Two bile acids

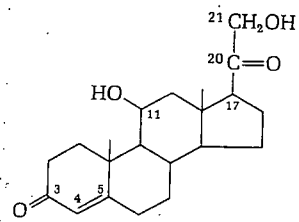


Cholic acid

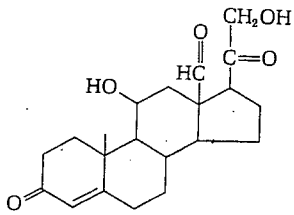


Deoxycholic acid

Two adrenocortical steroids

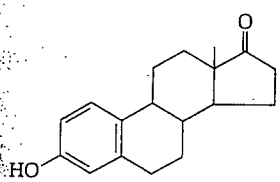


Corticosterone

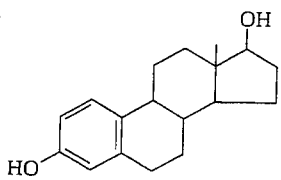


Aldosterone

Two estrogens (female sex hormones)

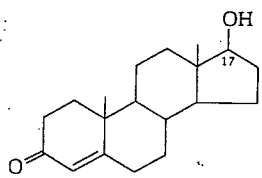


Estrone



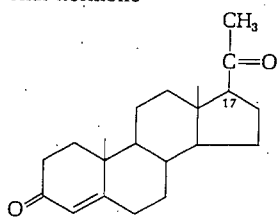
β-Estradiol

An androgen (male sex hormone)



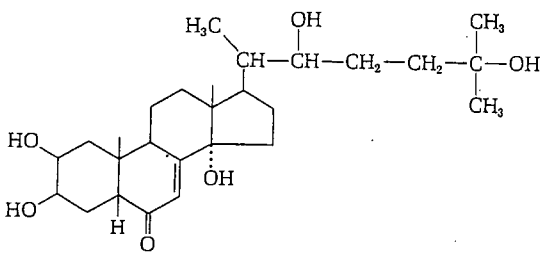
Testosterone

A progestational hormone



Progesterone

An insect molting hormone



Ecdysone

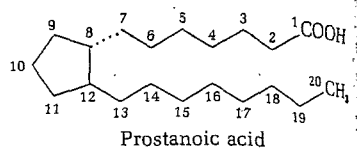
Prostaglandins

Prostaglandins are a family of fatty acid derivatives which have a variety of potent biological activities of a hormonal or regulatory nature. The name prostaglandin was first given in the 1930s by the Swedish physiologist U. S. von Euler to a lipid-soluble acidic substance found in the seminal plasma, the prostate gland, and the seminal vesicles. In very small amounts this material was found to lower blood pressure and to stimulate certain smooth muscles to contract. At first prostaglandin was thought to be a single substance, characteristically secreted by the male genital tract, but more recent research has shown that there are many different prostaglandins which function as regulators of metabolism in a number of tissues and in a number of ways. At least 14 prostaglandins occur in human seminal plasma, and many others have been found in other tissues or prepared synthetically in the laboratory.

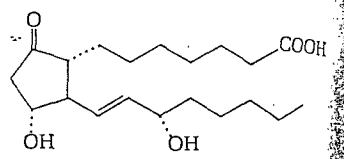
The structure of prostaglandins was established by S. Bergström and his colleagues in Sweden. All the natural prostaglandins are biologically derived by cyclization of 20-carbon unsaturated fatty acids, such as arachidonic acid, which is formed from the essential fatty acid linoleic acid (page 281). Five of the carbon atoms of the fatty acid backbone (carbons 8 through 12) are looped to form a five-membered ring (Figure 11-22). The prostaglandins are named according to their ring substituents and the number of additional side-chain double bonds, which have the cis configuration. The best known are prostaglandins E_1, $F_{1\alpha}$, and $F_{2\alpha}$, abbreviated as PGE_1, $PGF_{1\alpha}$, and $PGF_{2\alpha}$, respectively. These in turn are the parent compounds of further biologically active prostaglandins.

The prostaglandins differ from each other with respect to their biological activity, although all show at least some activity in lowering blood pressure and inducing smooth muscle to contract. Some, like PGE_1, antagonize the action of certain hormones. PGE_2 and $PGE_{2\alpha}$ may find clinical use in inducing labor and bringing about therapeutic abortion.

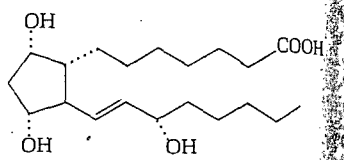
Figure 11-22
Some prostaglandins. The parent compound is prostanoic acid. The dotted bonds (color) project behind the plane of the page.



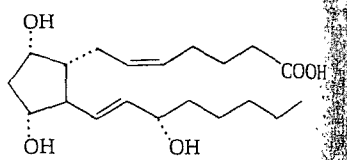
Prostanoic acid



Prostaglandin E_1 (PGE_1)



Prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$)



Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)

Lipid Micelles, Monolayers, and Bilayers

When a polar lipid, like a phosphoglyceride, is added to water, only a small fraction dissolves to form a true molecular solution. Above the *critical micelle concentration* the polar lipids associate into various types of aggregates resembling the micelles formed from soaps (page 43). In such structures (Figure 11-23) the hydrocarbon tails are hidden from the aqueous environment and form an internal hydrophobic phase whereas the hydrophilic heads are exposed on the surface. Triacylglycerols do not form such aggregates since they have no polar heads.

Phosphoglycerides also form monolayers on air-water interfaces as well as bilayers separating two aqueous compartments. Liposomes (Figure 11-23) are completely closed, vesicular bilayer structures formed by exposing phosphoglyceride-water suspensions to sonic oscillation. Bilayer systems of this sort have been extensively studied as models

ins. The parent compound
The dotted bonds (color)
plane of the page

5 3 1 COOH
4 2
3

20 CH_3
14 16 18
15 17 19

ostanoic acid

COOH
OH
glandin E_1 (PGE_1)

COOH
OH
glandin $F_{1\alpha}$ ($PGF_{1\alpha}$)

COOH
OH
glandin $F_{2\alpha}$ ($PGF_{2\alpha}$)

Figure 11-23
Stable phosphoglyceride-water systems.

Polar head
Nonpolar tails

Water

Micelles in water

Air

Water

Monolayer at air-water interface

Septum

Water

Aperture

Lipid bilayer

Phosphoglyceride bilayer in aperture separating two aqueous compartments

Water

Water

Water

Cross section through a liposome. The phospholipid bilayer forms a completely closed vesicle. Bilayers, like natural membranes, have self-sealing properties.

of natural membranes, which appear to contain polar phospholipid bilayers as their continuous phase (page 305).

Lipoprotein Systems

Certain lipids associate with specific proteins to form *lipoprotein systems* in which the specific physical properties of these two classes of biomolecules are blended. There are two major types, *transport lipoproteins* and *membrane systems*. In these systems the lipids and proteins are not covalently joined but are held together largely by hydrophobic interactions (page 43) between the nonpolar portions of the lipid and the protein components.

Transport Lipoproteins of Blood Plasma

The plasma lipoproteins are complexes in which the lipids and proteins occur in a relatively fixed ratio. They carry water-insoluble lipids between various organs via the blood, in a form with a relatively small and constant particle diameter and weight. Human plasma lipoproteins occur in four major classes that differ in density as well as particle size (Table 11-8). They are physically distinguished by their relative rates of flotation in high gravitational fields in the ultracentrifuge. All four lipoprotein classes have densities less than 1.21 g ml^{-1}, whereas the other plasma proteins, such as albumin and γ-globulin, have densities in the range 1.33 to 1.35 g ml^{-1}. The characteristic flotation rates in Svedberg flotation units (S_f) of the lipoproteins are determined in an NaCl medium of density 1.063 g ml^{-1} at 26°C, in which lipoproteins float upward and simple proteins sediment.

As shown in Table 11-8, the plasma lipoproteins contain varying proportions of protein and different types of lipid. The very low-density lipoproteins contain four different types of polypeptide chains having distinctive amino acid sequences. The high-density lipoproteins have two different types of polypeptide chains, of molecular weight 17,500 and 28,000. The polypeptide chains of the plasma lipoproteins are believed to be arranged on the surface of the molecules, thus conferring hydrophilic properties. However, in the very low-density lipoproteins and chylomicrons, there is insufficient protein to cover the surface; presumably the polar heads of the phospholipid components also contribute hydrophilic groups on the surface, with the nonpolar triacylglycerols in the interior.

Membranes

Membranes are a conspicuous feature of cell structure (pages 31 and 35); in some eukaryotic cells (page 33) the different membrane systems may make up as much as 80 percent of the total dry cell mass. Membranes serve not only as barriers separating aqueous compartments with different sol-

Table 11-8 Major classes of human plasma lipoproteins

	Chylomicrons	*Very low density lipoproteins (VLDL)*	*Low-density lipoproteins (LDL)*	*High-density lipoproteins (HDL)*
Density, g ml^{-1}	< 0.94	0.94–1.006	1.006–1.063	1.063–1.21
Flotation rate, S_f	> 400	20–400	0–20	(Sediment)
Particle size, nm	75–1,000	30–50	20–22	7.5–10
Protein, % of dry weight	1–2	10	25	45–55
Triacylglycerols, % of dry weight	80–95	55–65	10	3
Phospholipids, % of dry weight	3–6	15–20	22	30
Cholesterol, free, % of dry weight	1–3	10	8	3
Cholesterol, esterified, % of dry weight	2–4	5	37	15

ute composition but also as the structural base to which certain enzymes and transport systems are firmly bound. They are very thin (about 8 nm) and flexible.

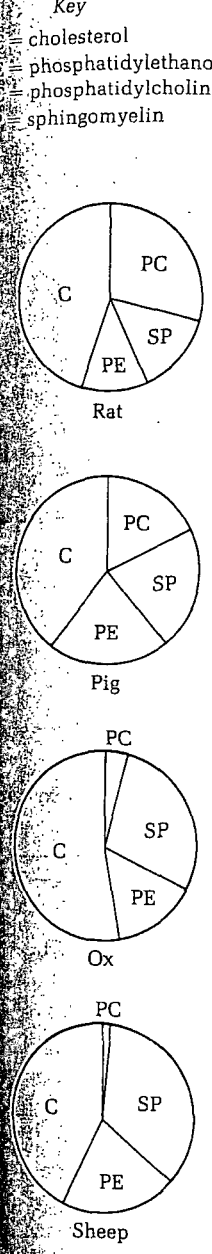
Membrane Components

Most membranes contain about 40 percent lipid and 60 percent protein, but there is considerable variation. At one extreme the inner mitochondrial membrane contains only about 20 to 25 percent lipid, and at the other the myelin membrane surrounding certain nerves may contain up to 75 percent lipid. The lipids of membranes are largely polar; phosphoglycerides predominate, with much smaller amounts of sphingolipids. In fact, nearly all the polar lipids of cells are localized in their membranes. Endoplasmic reticulum and organelle membranes contain relatively little cholesterol or triacylglycerol, whereas the plasma membrane of some cells of higher animals contains much cholesterol, both free and esterified. The ratio of different kinds of polar lipids in membranes is characteristic of the type of membrane system, the organ, and the species. As an example, Figure 11-24 shows the distribution of the major lipids in the plasma membranes of the erythrocytes of different animal species. The molar ratio of the different types of lipids in a membrane appears to be genetically determined; it cannot be altered, for example, by feeding vertebrates different mixtures of lipids. However, the fatty acid components of the individual lipids are not fixed and vary with nutritional state and environmental temperature. Within any given species of cell, the lipid composition of the different types of membranes are not necessarily identical; e.g., the lipid composition of the plasma membrane, the mitochondrial membrane, and the endoplasmic reticulum membrane of rat liver cells differs significantly.

Each type of membrane contains several or many kinds of proteins or polypeptides. Membrane proteins can be classified in two categories. The *extrinsic*, or *peripheral*, proteins are only loosely attached to the membrane surface and can easily be removed in soluble form by mild extraction procedures. The *intrinsic*, or *integral*, proteins, which make up 70 percent or more of the total membrane protein, are very tightly bound to the lipid portion and can be removed only by drastic treatment. The intrinsic proteins are highly insoluble in neutral aqueous systems but can be extracted by detergents such as sodium dodecyl sulfate or by unfolding agents such as 6 *M* guanidine hydrochloride. When the latter reagent was used to extract erythrocyte membranes, 17 different polypeptide chains having molecular weights from 27,000 to 220,000 were obtained. Among them is *glycophorin*, a glycoprotein that extends completely across the membrane. The inner mitochondrial membrane is one of the most complex membranes; it probably contains over 100 different kinds of polypeptide chains.

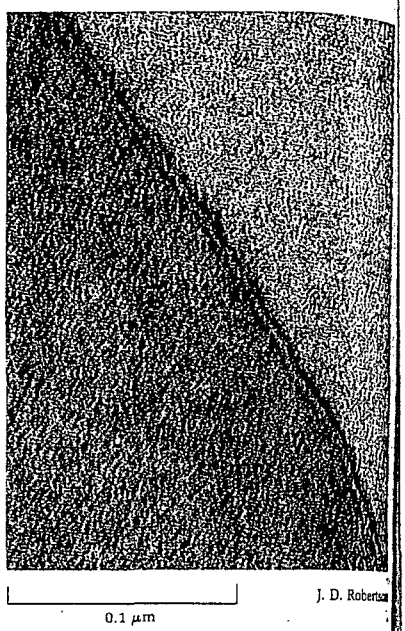
Various physical methods have been used to ascertain the arrangement of the lipid and protein molecules in the structure of membranes. Electron microscopy has revealed that

Figure 11-24
Lipid composition of erythrocyte membranes of different mammals. Note that the proportions of cholesterol and phosphatidylethanolamine are approximately constant but that the ratio of phosphatidylcholine to sphingomyelin varies greatly with the species.



membranes have a trilaminar structure (Figure 11-25) with a total thickness between 7.0 and 9.0 nm, depending on the type of membrane. Optical-rotatory-dispersion and circular-dichroism measurements (page 150) indicate that the protein molecules in membranes have a rather high content of right-handed α helix. The lipid molecules of membranes appear to be arranged in a bilayer structure. This follows not only from the high electrical resistance of natural membranes, which indicates the presence of a continuous hydrocarbon phase, but also from experiments with the spin-label technique. In this ingenious method, first developed by H. McConnell and his colleagues, a fatty acid or lipid bearing a *spin label* is incorporated into the membrane. A widely used spin label is the nitroxyl group (Figure 11-26); its N → O bond contains an unpaired electron whose spin has a specific direction in relation to the long axis of the fatty acid chain. The directional orientation and motion of the spin-labeled fatty acid or lipid in a bilayer or membrane can be determined by *electron spin-resonance spectroscopy* (ESR), also called *electron paramagnetic-resonance spectroscopy* (EPR), which detects the paramagnetism generated by the unpaired electron spin. With this method it has been shown that the phospholipids in natural membranes move freely in the plane of the membrane but do not cross readily from one side to the other. Moreover, by placing the spin label at different distances along the hydrocarbon chain of a fatty acid molecule it has been found that the nonpolar end of the chain wiggles readily, whereas the end near the carboxyl group is relatively rigid, nearly perpendicular to the plane of the membrane.

Figure 11-25
High-magnification electron micrograph of erythroctye plasma membrane, showing the trilaminar image yielded by fixation in osmium tetroxide. Usually the two electron-dense lines separate a clear space of about 2.5 nm.

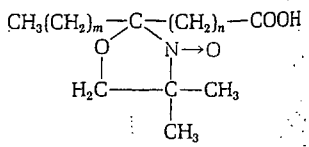


Membrane Structure

The first important hypothesis of the structure of biological membranes was proposed by H. Davson and J. Danielli in 1935. An important feature of their hypothesis is the proposal that membranes contain a continuous hydrocarbon phase contributed by the lipid components of the membrane. Some years later this hypothesis was modified and refined, particularly by J. D. Robertson, into the *unit-membrane hypothesis*. The unit membrane was proposed to consist of a bilayer of mixed polar lipids, with their hydrocarbon chains oriented inward to form a continuous hydrocarbon phase and their hydrophilic heads oriented outward. Each surface was thought to be coated with a monomolecular layer of protein molecules, with the polypeptide chains in extended form. The total thickness of the unit membrane was suggested to be about 8.0 to 9.0 nm, the thickness of the lipid bilayer about 6.0 to 7.0 nm. Later other investigators proposed *globular* or *subunit* models, in which membranes were viewed as consisting of sheets of recurring lipoprotein subunits of diameter 4.0 to 9.0 nm, resembling the subunit structure of some oligomeric proteins or the coats of some viruses (page 62). However, globular models have failed to account satisfactorily for many properties of membranes.

The most satisfactory model of membrane structure to date appears to be the *fluid-mosaic* model, postulated by S. J.

Figure 11-26
A fatty acid bearing the nitroxyl group as a spin label.



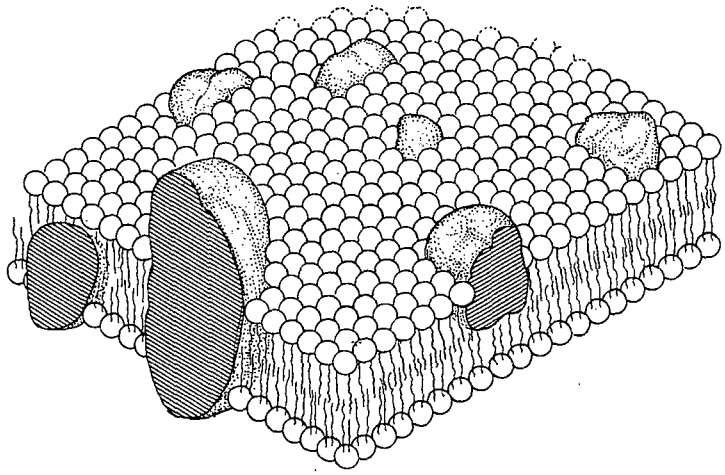


Figure 11-27
The fluid-mosaic model of membrane structure. The membrane consists of a fluid phospholipid bilayer with globular protein molecules penetrating into either side or extending entirely through the membrane. There is no long-range regularity in the spacing of the protein molecules, but some may be organized into complexes. Presumably the membrane is asymmetric. The outer surface of the plasma membrane of eukaryotic cells has oligosaccharide chains protruding from glycolipids and glycoproteins (see Figure 10-36, page 275). [Modified from S. J. Singer and G. L. Nicolson, Science, 175:720–731 (1972).]

Singer and G. L. Nicolson in 1972. This model (Figure 11-27) postulates that the phospholipids of membranes are arranged in a bilayer to form a fluid, liquid-crystalline matrix or core. In this bilayer individual lipid molecules can move laterally, endowing the bilayer with fluidity, flexibility, and a characteristically high electrical resistance and relative impermeability to highly polar molecules. The fluid-mosaic model postulates that the membrane proteins are globular, to account for their high content of α helix. Some of the proteins are partially embedded in the membrane, penetrating into the lipid phase from either side, and others completely span the membrane. To what extent a given globular protein penetrates into the lipid phase would be determined by the amino acid sequence of the protein and the location on its surface of the nonpolar amino acid R groups. Thus the various membrane proteins would form a mosaiclike structure in the otherwise fluid phospholipid bilayer. This mosaic is not fixed or static, since the proteins are free to diffuse laterally in two dimensions, at least in some membranes. The relative viscosity of the lipid bilayer is thought to be from 100 to 1,000 times that of water.

The fluid-mosaic model accounts satisfactorily for many features and properties of biological membranes. It provides for membranes with widely different protein content, depending on the number of different protein molecules per unit area of membrane; it provides for the varying thickness of different types of membranes; it can account for the asymmetry of natural membranes, since it permits proteins of different types to be arranged on the two surfaces of the lipid bilayer; it accounts for the electrical properties and permeability of membranes; and it also accounts for the observation that some protein components of cell membranes move in the plane of the membrane at a rather high rate.

Most membranes appear to be asymmetric, with different types and/or numbers of specific proteins on each surface. In particular, the plasma membrane of eukaryotic cells often has an outer coat or *glycocalyx* (page 271) made up of the hydrophilic oligosaccharide side chains of membrane glycoproteins and the oligosaccharide head groups of membrane glycolipids.

Summary

Lipids are water-insoluble components of cells that can be extracted by nonpolar solvents. The complex, or saponifiable, lipids contain fatty acids, usually with an even number of carbon atoms, 12 to 22 carbon atoms long. The double bonds of unsaturated fatty acids usually have the cis configuration. In most unsaturated fatty acids, one double bond is at the 9,10 position. Fatty acids can be separated and analyzed by gas-liquid partition chromatography.

Triacylglycerols (triglycerides) contain three fatty acid molecules esterified to the three hydroxyl groups of glycerol. Triacylglycerols serve primarily to store fuel in the form of fat droplets in cells. The phosphoglycerides contain two fatty acid molecules esterified to the two free hydroxyl groups of glycerol 3-phosphate and an alcohol esterified to the phosphoric acid. Their polar head groups differ in polarity and charge. They occur mainly in membranes. Sphingolipids contain no glycerol but have two long hydrocarbon chains, one contributed by a fatty acid and the other by sphingosine, a long-chain aliphatic amino alcohol. Sphingomyelin is the only sphingolipid containing phosphoric acid. The neutral glycosphingolipids contain a carbohydrate head group; cerebrosides, the simplest, contain either D-glucose or D-galactose. Gangliosides are acidic glycosphingolipids containing one or more residues of *N*-acetylneuraminic acid; they are important elements in cell surfaces. Waxes are fatty acid esters of high-molecular-weight alcohols.

The simple, or nonsaponifiable, lipids include the terpenes and the steroids. Terpenes are linear or cyclic compounds built of two or more isoprene units. Steroids are derived from the terpene squalene. The sterols are steroid alcohols; cholesterol is the most abundant sterol in animal tissues. Other steroids include sex hormones, adrenocortical hormones, and bile acids. The prostaglandins, cyclic derivatives of 20-carbon unsaturated fatty acids, function in biological regulation.

Polar lipids spontaneously form micelles, monolayers, and bilayers. Lipids are transported in the blood by the plasma lipoproteins, of which there are four different classes differing in density.

Most membranes contain about 50 to 60 percent protein and 40 to 50 percent lipid. The lipids are present in fixed molar ratios, which are probably genetically determined. Several models of membrane structure have been proposed. Much evidence supports the fluid-mosaic model, which consists of a liquid-crystalline phosphoglyceride bilayer, into which globular proteins penetrate partially or completely. Some plasma membrane proteins contain oligosaccharide side chains, which protrude from the cell surface.

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Problems

1. Indicate the structures of all possible isomers of a triacylglycerol containing palmitic, stearic, and oleic acids. Include both positional isomers and stereoisomers.

2. A mixture of triacylglycerols when hydrolyzed yielded oleic (O), palmitic (P), and stearic (S) acids.
   (*a*) Indicate the structure of all possible molecular species in the original mixture.
   (*b*) If only L-stereoisomers are present in the mixture, how many species would be present?

3. A sample (5 g) of the triglycerides extracted from an avocado required 36.0 ml of 0.5 *M* KOH for complete hydrolysis and conversion of its fatty acids into soaps. Calculate the average chain length of the fatty acids in the sample.

4. A mixture of 1-palmitoyl-2-stearoyl-3-lauroylglycerol and phosphatidic acid in benzene is shaken with an equal volume of water. After the two phases are allowed to separate, which lipid will be in higher concentration in the aqueous phase? Why?

5. Electrophoresis at pH 7.0 was carried out on a mixture of lipids containing (*a*) cardiolipin, (*b*) phosphatidylglycerol, (*c*) phosphatidylethanolamine, (*d*) phosphatidylserine, and (*e*) *O*-lysylphosphatidylglycerol. Indicate how you would expect these compounds to move: toward the anode (A), toward the cathode (C), or remain at origin (O).

6. Name the products of hydrolysis with dilute sodium hydroxide of (*a*) 1-stearoyl-2,3-dipalmitoylglycerol, (*b*) 1-stearoyl-2-elaidoylphosphatidylinositol, (*c*) 1-palmitoyl-2-oleyl phosphatidylcholine.

7. Name the products of the following: (*a*) hydrolysis of 1-stearoyl-2-oleyl phosphatidylserine by strong base, followed by acid hydrolysis, (*b*) treatment of 1-palmitoyl-2-linoleyl phosphatidylcholine with phospholipase D.

8. Most membranes of animal cells contain about 60 percent by weight of protein and 40 percent by weight phosphoglycerides. (*a*) Calculate the average density of a membrane, assuming that protein has a density of 1.33 g cm^{-3} and phosphoglyceride a density of 0.92 g cm^{-3}.
(*b*) If a sample of membrane material were centrifuged in NaCl solution of density 1.05 g cm^{-3}, would it sediment or float?

9. If a membrane contains 60 percent by weight of proteins and 40 percent of phosphoglycerides, calculate the molar ratio of phosphoglyceride to protein. Assume that the lipid molecules have an average molecular weight of 800 and the proteins an average molecular weight of 50,000.

For Jan

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13

METABOLISM OF FATTY ACIDS AND TRIACYLGLYCEROLS

Lipids are broadly defined as biological molecules that are soluble in organic solvents. Although the lipids encompass a large and diverse group of compounds, they have only four major biological functions: (1) in all cells, the major structural elements of the membranes are composed of lipids; (2) certain lipids (the *triacylglycerols*) serve as efficient reserves for the storage of energy; (3) many of the vitamins and hormones found in animals are lipids or derivatives of lipids, and (4) the bile acids help to solubilize the other lipid classes during digestion.

This and the following two chapters present an introduction to the biochemistry of the lipids. The subject has been somewhat artificially divided. The advantage of such a division is to package the subject into a smaller and more easily digestible format. Obviously, this division does not occur in cells and organisms. The metabolism of fatty acids, phospholipids, triacylglycerols, and steroids impinge on one another. Similarly, the metabolism of carbohydrates has striking effects on lipid metabolism. With this caveat in mind, let us begin our discussion of fatty acids.

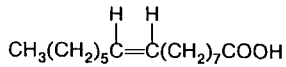
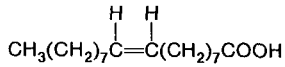
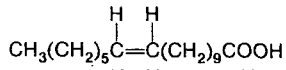
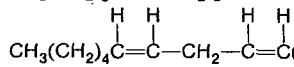
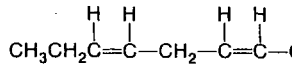
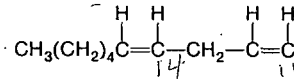
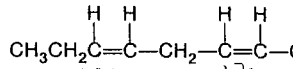
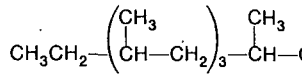
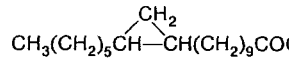
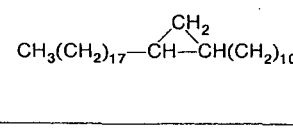
FATTY ACIDS

Structure of Fatty Acids

Compounds with the structural formula $CH_3(CH_2)_nCOOH$ that contain no carbon–carbon double bonds are known as *saturated fatty acids*. The two most abundant saturated fatty acids are *palmitic* and *stearic* acids

Space-filling model of triacylglycerol, a most efficient molecule for storing chemical energy.

Table 13-1
Fatty Acids

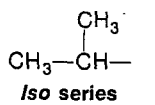
Common Name	Systematic Name	Structure	Abbreviation
Saturated fatty acids			
Myristic acid	*n*-Tetradecanoic acid	$CH_3(CH_2)_{12}COOH$	14:0
Palmitic acid	*n*-Hexadecanoic acid	$CH_3(CH_2)_{12}CH_2CH_2COOH$	16:0
Stearic acid	*n*-Octadecanoic acid	$CH_3(CH_2)_{12}CH_2CH_2CH_2CH_2COOH$	18:0
Arachidic acid	*n*-Eicosanoic acid	$CH_3(CH_2)_{12}CH_2CH_2CH_2CH_2CH_2CH_2COOH$	20:0
Behenic acid	*n*-Docosanoic acid	$CH_3(CH_2)_{12}CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COOH$	22:0
Lignoceric acid	*n*-Tetracosanoic acid	$CH_3(CH_2)_{12}CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COOH$	24:0
Cerotic	*n*-Hexacosanoic acid	$CH_3(CH_2)_{12}CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COOH$	26:0
Unsaturated fatty acids			
Palmitoleic acid	*cis*-9-Hexadecenoic acid		$16:1^{\Delta 9}$
Oleic acid	*cis*-9-Octadecenoic acid		$18:1^{\Delta 9}$
Vaccenic acid	*cis*-11-Octadecenoic acid		$18:1^{\Delta 11}$
Linoleic acid	*cis,cis*-9,12-Octadecadienoic acid		$18:2^{\Delta 9,12}$
α-Linolenic acid	All-*cis*-9,12,15-Octadecatrienoic acid		$18:3^{\Delta 9,12,15}$
Arachidonic acid	All-*cis*-5,8,11,14-Eicosatetraenoic acid		$20:4^{\Delta 5,8,11,14}$
	All-*cis*-4,7,10,13,16,19-Docosahexaenoic acid		$22:6^{\Delta 4,7,10,13,16,19}$
Some unusual fatty acids			
	2,4,6,8-Tetramethyl decanoic acid		
Lactobacillic acid			
An α-mycolic acid			

(Table 13-1). Some other saturated fatty acids present in smaller quantities in mammalian tissues also are shown in Table 13-1. The *sphingolipids*, which will be discussed in Chapter 14, contain longer-chain fatty acids (n = 20–24), as well as palmitic and stearic acids. In some tissues, short-chain fatty acids also are found, such as decanoic acid (10:0) in milk.

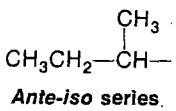
Fatty acids with double bonds in the aliphatic chain are called unsaturated fatty acids. *Monounsaturated* fatty acids have one double bond, and *polyunsaturated fatty acids* contain more than one double bond. The double bonds in virtually all naturally occurring fatty acids are cis. Fatty acids are often abbreviated as shown in Table 13-1. The number to the left of the colon indicates the number of carbon atoms of the fatty acid and the number to the right indicates the number of double bonds. The numbering begins from the carboxyl group. The position of the double bond is shown by a superscript Δ followed by the number of carbons between the double bond and the carboxyl group. The double bonds in polyunsaturated fatty acids are always separated by one methylene group. Mammalian tissues contain all the unsaturated fatty acids listed in Table 13-1 with the exception of vaccenic acid, which is present in *Escherichia coli* and other bacteria. *E. coli* does not contain polyunsaturated fatty acids. Oleic acid is the most common monounsaturated fatty acid in mammals. Two unsaturated fatty acids, *linoleic* and *linolenic* acids, are not synthesized by mammals and are therefore important dietary requirements. Like vitamins, these two fatty acids are required for growth and good health. Hence they are called *essential fatty acids*. Plants are able to synthesize linoleic and linolenic acids and are the original source of these fatty acids in our diet.

Sometimes unsaturated fatty acids are numbered from the terminal methyl group. In this instance, the numbering is preceded by a lowercase omega (ω). Thus linoleic acid might be called Δ9,12-octadecadienoic acid or ω-6,9-octadecadienoic acid.

In addition to the commonly occurring fatty acids, many structural variations have evolved. There are well over 100 other fatty acids found in various creatures and organisms, often associated with specialized functions. For instance, branched-chain fatty acids are found in many different tissues. The uropygial gland of the duck produces such a fatty acid (2,4,6,8-tetramethyldecanoic acid). The duck uses the fatty acids secreted by this gland to preen its feathers and thereby ensure that water continues to "run off its back." In the bacterial genus *Bacillus*, the monoenoic fatty acids are replaced by branched-chain fatty acids in which a methyl group is adjacent to either the terminal methyl group (the *iso* series) or the terminal ethyl group (the *ante-iso* series). Another example is fatty acids with a *cyclopropane ring* in the alkyl chain found in many bacteria. The bacterium that causes the disease tuberculosis, *Mycobacterium tuberculosis*, produces a family of complex fatty acids known as *mycolic acids*, which contain cyclopropane rings. One class of these is the α-mycolic acids and an example is given in Table 13-1. Many structurally related α-mycolic acids are found in the mycobacteria and other related bacteria (nocardiae and corynebacteria). These mycolic acids appear to have a structural function in the outer part of the bacterial cell wall. There is much evidence to suggest that a major drug used in the treatment of tuberculosis (Isoniazid) functions by the inhibition of an early reaction of α-mycolic acid biosynthesis.



***Iso* series**



***Ante-iso* series**

Properties and Analysis of Fatty Acids

Fatty acids are usually found as components of complex lipids, and hence, only a very small percent exists as unesterified (free) fatty acids. Nevertheless, it is worth noting that the pK_a for dissociation of the acid proton is around 4.7. Therefore, at pH 7.0, the fatty acid exists primarily in the dissociated form ($RCOO^-$):

$$CH_3(CH_2)_nCOOH \rightleftharpoons CH_3(CH_2)_nCOO^- + H^+$$

Because it exists as a salt at neutral pH, it is not easily extracted from an aqueous medium by organic solvents such as hexane. However, if the pH is lowered by the addition of HCl or another strong acid, the fatty acid becomes protonated and is easily extracted by organic solvents.

Another property of fatty acids that should be noted is the physical form of the fatty acids at room temperature. If n is equal to 8 or less, the fatty acid is a liquid, whereas if n is equal to 10 or more, the fatty acid is a solid. If a fatty acid has a double bond, it has a lower melting point than the saturated fatty acid with the same number of carbons. Unsaturated fatty acids are more condensed in length than the corresponding saturated fatty acids (Figure 13-1). The double bonds prevent the tight packing within membranes that occurs with saturated fatty acids (see Chapter 16).

When fatty acids were first discovered and chemists were involved in structural determinations, a major problem was separation of the various fatty acids into pure compounds. Consider the difficulties that were encountered in the separation of stearic acid from palmitic acid by the tech-

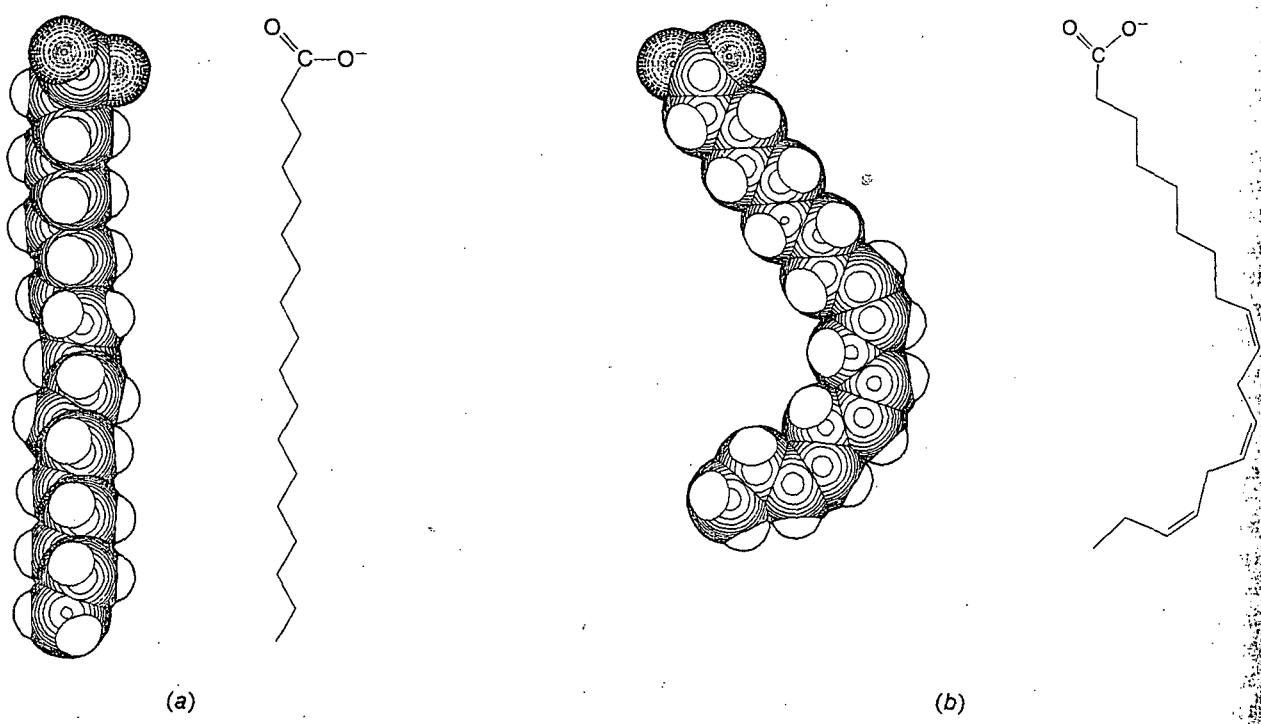


Figure 13-1
Space-filling model of stearic and linolenic acids.

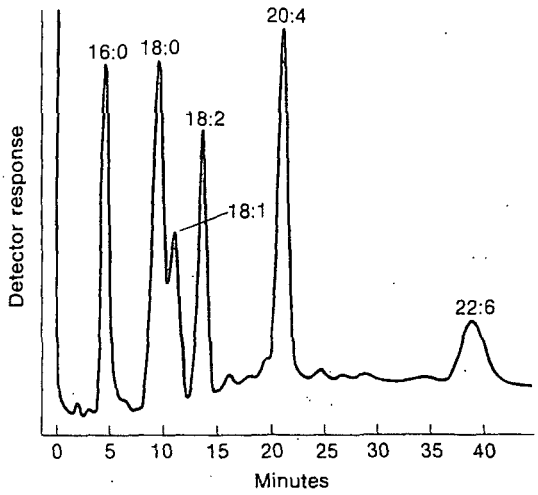


Figure 13-2
Gas chromatogram of methyl esters of fatty acids derived from total lipids of rat liver. The analysis was done on a 6-ft column packed with 15% ethylene glycol succinate on 80/100 Gas Chrom P. The temperature was 150°C for 15 min, programmed to 180°C at 6°C per min and held at 180° until 22:6 had eluted.

niques available in the early 1900s (organic extractions and crystallization). However, with the advent of gas-liquid chromatography in the 1950s, it became relatively easy to analyze and purify fatty acids from complex mixtures. Today fatty acids are commonly analyzed by gas chromatography of the methyl esters. These are formed by esterification of the fatty acids with methanol [R represents hydrogen (H) or any group to which the fatty acid is esterified]:

$$CH_3(CH_2)_n\overset{O}{\overset{\|}{C}}—OR + CH_3OH \xrightarrow[HCl \text{ or } BF_3]{} CH_3(CH_2)_n\overset{O}{\overset{\|}{C}}OCH_3 + ROH$$

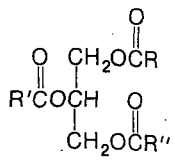
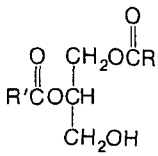
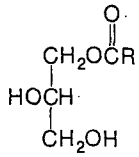
An analysis of the fatty acids from rat liver is shown in Figure 13-2.

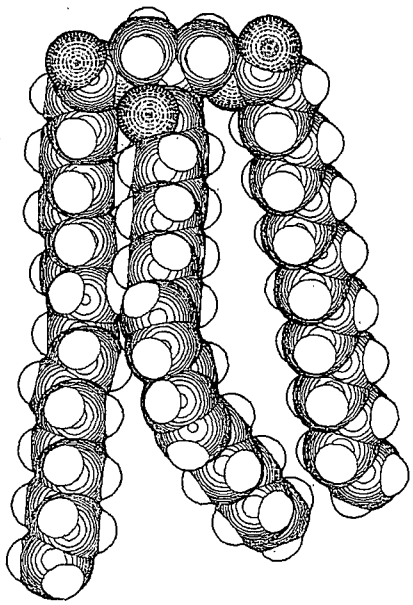
Triacylglycerols (Triglycerides)

Fatty acids are major components of the *triacylglycerols* (Table 13-2 and Figure 13-3) and most of the complex lipids present in membranes. *It is in the triacylglycerols that fatty acids are stored as an energy reserve*. Triacylglycerols are the major uncharged glycerol derivatives found in animals. The monoacylglycerols and diacylglycerols are metabolites of triacylglycerols (and of phospholipids, as discussed in Chapter 14) and are normally present in cells in very small quantities.

Because the substituents esterified to the first and third carbons are usually different, the second carbon of the glycerol derivative is asymmetric. In naming and numbering these glycerol derivatives, a special convention has been adopted: The prefix *sn-* (for *s*tereospecifically *n*umbered) immediately precedes "glycerol" and differentiates the naming of the compound from other approaches, such as the *RS* system described in Chapter 6. The glycerol derivative is drawn in a Fischer projection with the secondary hydroxyl to the *left* of the central carbon, and the carbons are numbered 1, 2, and 3 from the top to the bottom. The prefix *rac-* (for *racemo*) precedes the name if the compound is an equal mixture of antipodes. If the configuration is unknown or not specified, x- precedes the name.

Table 13-2
Neutral Glycerides

Common Name	Systematic Name	Structure
Triglyceride	1,2,3-Triacyl-*sn*-glycerol	
Diglyceride	1,2-Diacyl-*sn*-glycerol	
Monoglyceride	1-Monoacyl-*sn*-glycerol	



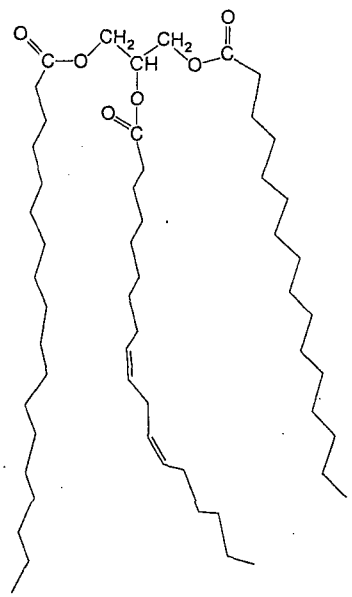


Figure 13-3
Space-filling model of triacylglycerol.

Although triacylglycerols are found in the liver and intestine, they are primarily found in adipose tissue (fat), which functions as a storage depot for this lipid. The specialized cell in this tissue is called the *adipocyte*. The cytoplasm of the cell is full of lipid vacuoles that are almost exclusively triacylglycerols (Figure 13-4) and serve as an energy reserve for mammals. At times when the diet or glycogen reserves are insufficient to supply the body's need for energy, the fuel stored as fatty acyl components of the triacylglycerols is mobilized and transported to other tissues in the body. A second important function of adipose tissue is insulation of the body from cold. This function is most obvious in such cold-water mammals as the arctic whales (Beluga whales), which have vast stores of fat (blubber).

MOBILIZATION AND TRANSFER OF FATTY ACIDS FROM ADIPOSE TISSUE

Mobilization of Fatty Acids from Triacylglycerols

When the energy supply from diet becomes limited, the animal responds to this deficiency with a hormonal signal that is transmitted to the adipose tissue by the release of *epinephrine*, *glucagon*, and other hormones. The hormones bind to the plasma membrane of the adipocyte and stimulate the synthesis of *cyclic AMP* (cAMP), as previously discussed for the mobilization of glycogen (Chapter 8). As shown in Figure 13-5, this process involves the activation by cAMP of a protein kinase that phosphorylates and activates hormone-sensitive *triacylglycerol lipase*. The latter enzyme hydrolyzes the triacylglycerol to diacylglycerol with release of a fatty acid from carbon 1 or 3 of the glycerol backbone. This reaction is thought to be the rate-limiting step in the complete hydrolysis of the triacylglycerols. The diacylglycerols and monoacylglycerols are rapidly hydrolyzed to yield fatty

acids and glycerol. At this juncture it is not clear whether the diacylglycerol lipase is a separate enzyme or the same enzyme as triacylglycerol lipase. However, monoacylglycerol lipase is a separate enzyme, at least in chicken liver.

The unesterified fatty acids move through the plasma membranes of the adipocytes and endothelial cells of the blood capillaries and are bound by *albumin* in plasma. The mechanism for transfer of these fatty acids from inside the adipocytes to the plasma compartment is thought to involve passive diffusion. Hence the rate of transfer depends on the concentrations of fatty acids both in the adipocytes and in the plasma. Albumin carries the fatty acids to other tissues in the body. The glycerol also can be released into the plasma and be taken up by the liver for glucose production.

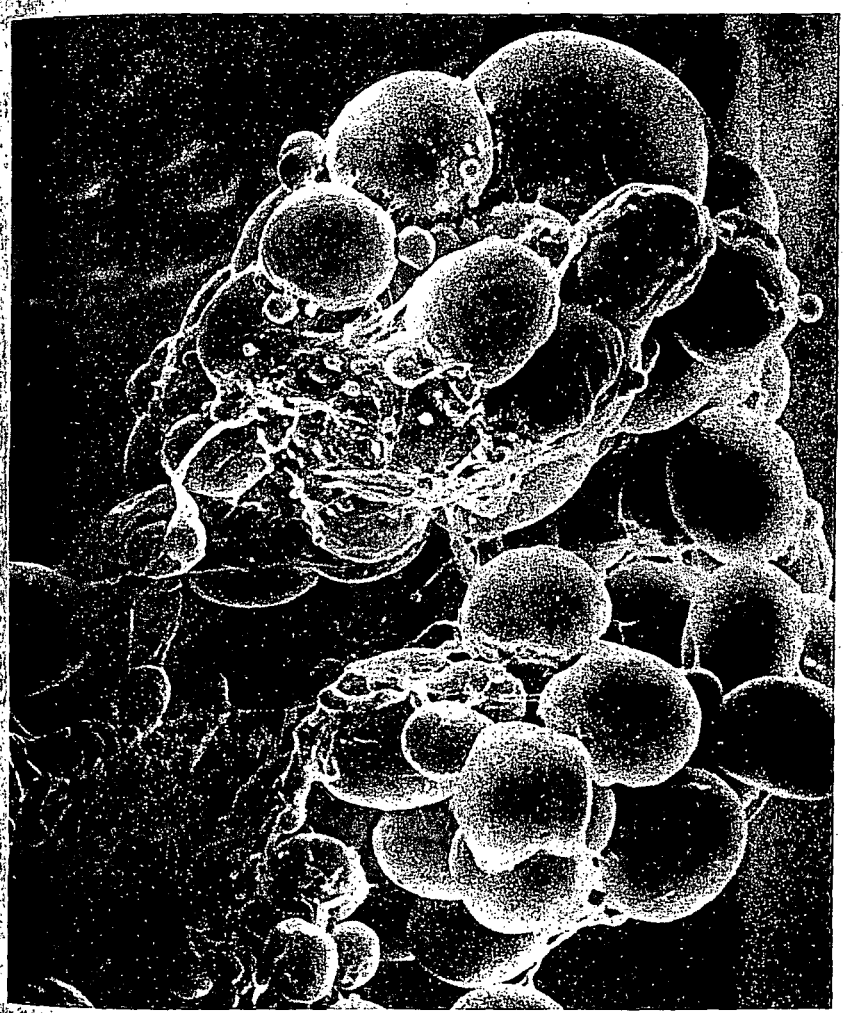


Figure 13-4
Scanning electron micrograph of white adipocytes from rat adipose tissue (500 ×). (Courtesy Dr. A. Angel and Dr. M. J. Hollenberg of the University of Toronto.)

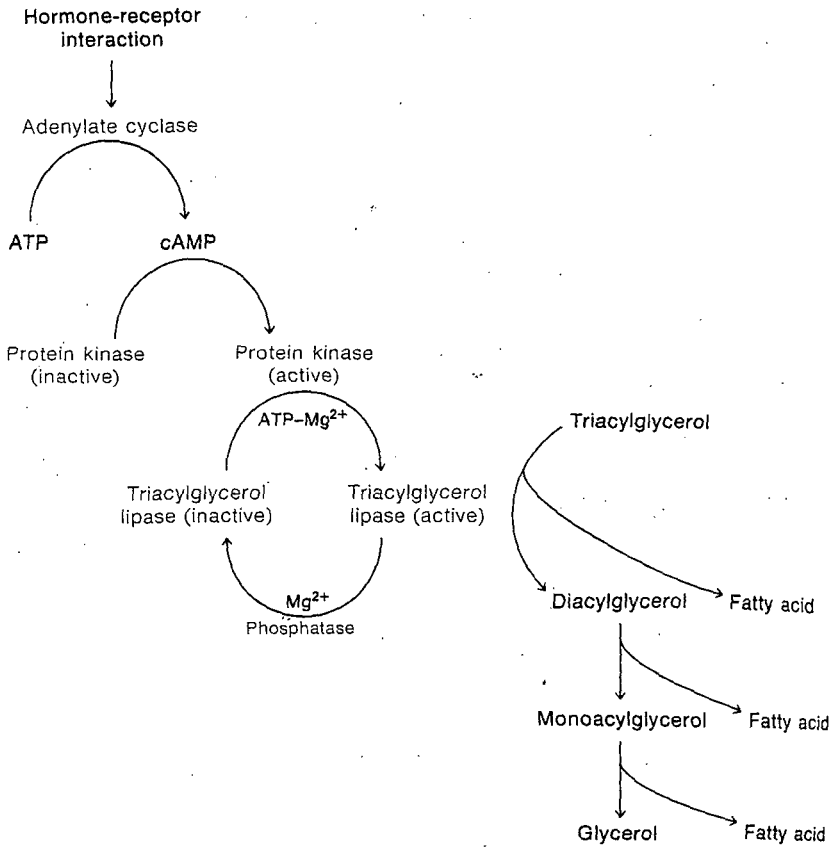


Figure 13-5
Activation of triacylglycerol lipase in adipose tissue.

Albumin: A Principal Carrier of Fatty Acids in the Plasma

Albumin is a quantitatively significant protein in humans because it constitutes about 50 percent (4 g/dl) of the plasma proteins. The protein has a molecular weight of 66,200 and is a single polypeptide chain that is linked together by 17 disulfide bridges. Each albumin molecule has the capacity to bind 10 molecules of fatty acid, although normally only 0.5 to 2 mol of fatty acid is bound. There appear to be two or three primary binding sites. Longer-chain fatty acids bind more readily to albumin than do shorter-chain fatty acids (e.g., stearate binds more readily than palmitate). Monounsaturated fatty acids bind with higher affinity to albumin than saturated fatty acids (e.g., oleate binds more readily than stearate). However, linoleate binds less readily than stearate. The time (half-life) it takes one-half the fatty acids bound to albumin in plasma to be taken up by various tissues is 1 to 2 min.

Although albumin has long been considered an essential protein of plasma, a few people with depressed levels of albumin (4.6 to 24 mg/dl compared with 4 g/dl) have been described (*analbuminemia*). Curiously,

these people are virtually asymptomatic and their problem is usually diagnosed as a result of routine blood analyses. Clearly, large quantities of albumin are not essential for life. The transport function of albumin in people suffering from analbuminemia is most likely assumed by the lipoproteins. *Albumin carries the fatty acids to energy-deficient tissues, where the fatty acids move from the plasma compartment into the tissues by a process of diffusion.* The amount of fatty acid removed by a tissue depends on the relative concentrations both in the plasma and in the cells of the tissues. Cardiac and red muscle utilize fatty acids as the major oxidative source of adenosine triphosphate (ATP) and therefore remove large amounts from the circulation.

Acyl-CoA Synthases: The Enzymes that Activate Fatty Acids

The fatty acids that are taken into the cells are activated in the cytosol by reaction with *coenzyme A* (CoA) and ATP to yield fatty acyl-CoA in a reaction catalyzed by *acyl-CoA synthase* (also known as *thiokinase*):

$$RCOO^- + ATP + CoA \overset{Mg^{2+}}{\rightleftharpoons} RCO{-}CoA + PP_i + AMP$$

In addition to the cytosolic enzyme, there are several synthases that activate fatty acids in the mitochondria and at least one thiokinase associated with microsomes. As is the case with other reactions in which PP_i is a product, this is rapidly hydrolyzed to 2 P_i, and therefore, the formation of the fatty acyl-CoA derivative is highly favored. Hence two high-energy bonds are hydrolyzed for the synthesis of one acyl-CoA. The concentration of PP_i in the cells (0.01 m*M* in rat liver) is very low; thus the pyrophosphorolysis of the acyl-CoA by the synthase is prevented.

Transport of Fatty Acids into the Mitochondria

Fatty acids cannot be utilized for the energy requirements of cells until they have been transported into the mitochondria, the major sites of β oxidation. *Fatty acyl-CoAs, which cannot cross the inner membranes of mitochondria, are converted to their acyl carnitine derivatives, which can cross this membrane:*

$$RCO{-}CoA + (CH_3)_3\overset{+}{N}{-}CH_2CH(OH)CH_2COO^- \rightleftharpoons$$

Carnitine

$$(CH_3)_3\overset{+}{N}{-}CH_2CH(O{-}C(=O)R)CH_2COO^- + CoA$$

Acyl carnitine

This reaction is catalyzed by *carnitine acyltransferase I*. There are at least three acyltransferases associated with mitochondria: one specific for short-chain fatty acids (*carnitine acetyltransferase*) and two specific for the longer-chain fatty acids (*carnitine acyltransferases I and II*). There also is evidence for an acyltransferase with intermediate-chain-length specificity.

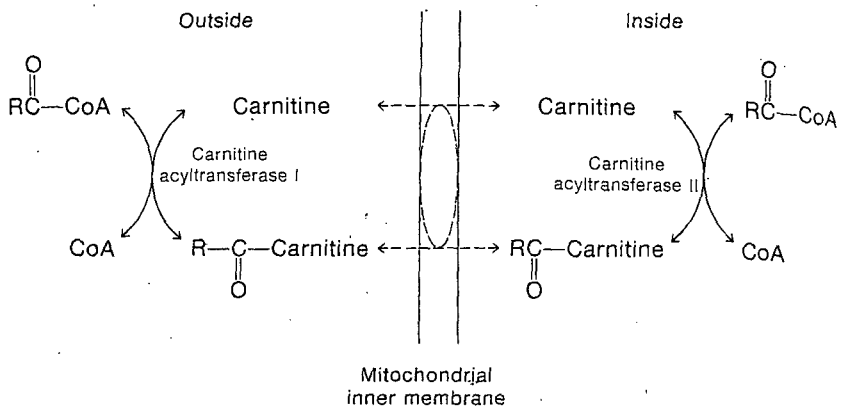


Figure 13-6
Transport of acyl derivatives across the mitochondrial membrane.

There is a protein carrier in the inner mitochondrial membrane that can transport carnitine, acetyl-carnitine, and short- and long-chain acyl carnitine derivatives across the membrane. The transfer of fatty acyl carnitine into the mitochondria appears to involve an exchange with free carnitine, as illustrated in Figure 13-6. Once inside the mitochondria, the reaction is reversed by *carnitine acyltransferase II* to yield a fatty acyl-CoA inside the mitochondria. Thus there are at least two distinct pools of acyl-CoA in the cell, one in the cytosol and the other in the mitochondria.

OXIDATION OF FATTY ACIDS

Historical Background

Our current understanding of fatty acid oxidation did not start to develop in detail until the early 1950s. However, in 1905, F. Knoop reported a series of experiments that indicated that fatty acids were oxidized by removal of two carbons at a time. He fed rabbits fatty acids in which the methyl group had been replaced with a phenyl group. If the altered fatty acid contained an even number of carbons [for example, $C_6H_5—CH_2(CH_2)_2COOH$], the primary metabolite was phenylacetic acid ($C_6H_5—CH_2COOH$), which was excreted as its glycine conjugate, phenylaceturic acid ($C_6H_5—CH_2CO—NHCH_2COOH$). When he fed the rabbits a phenyl derivative of a fatty acid with an odd number of carbons [for example, $C_6H_5—CH_2(CH_2)_3COOH$], he found benzoic acid (C_6H_5COOH), which was excreted in the urine as its glycine conjugate, hippuric acid ($C_6H_5CO—NH—CH_2COOH$). Knoop postulated that *fatty acids were oxidized at the β carbon (hence β oxidation) and degraded to acetic acid and a fatty acid with two fewer carbons:*

$$RCH_2CH_2COOH \longrightarrow R\overset{O}{\overset{\|}{C}}CH_2COOH \longrightarrow R\overset{O}{\overset{\|}{C}}OH + CH_3COOH$$

The next major experimental step was the demonstration in 1944 by Luis LeLoir that fatty acids could be oxidized in a cell-free system. This was followed by Albert Lehninger's demonstration that the process of fatty acid oxidation occurred in liver mitochondria and, apparently, involved an

"active acetate." Experiments by Fritz Lipmann proved that CoA was involved in the formation of "active acetate":

$$\text{Acetate} + \text{ATP} + \text{CoA} \longrightarrow \text{"active acetate"}$$

Subsequently, in 1951, Feodor Lynen, working in Munich with yeast, demonstrated that "active acetate" was acetyl-CoA. At this stage, several laboratories conceived the idea that CoA may play a role in the activation of fatty acids for β oxidation, and by 1954, the basic outline of β oxidation as we know it today was developed.

Overall Scheme for β Oxidation, the Principal Route for Catabolism of Fatty Acids

The outline for β oxidation of a saturated fatty acid is shown in Figure 13-7. In the first reaction, the acyl-CoA is dehydrogenated to yield the α–β(or 2–3) *trans*-enoyl-CoA and $FADH_2$. This enoyl-CoA is subsequently hydrated stereospecifically to yield the 3-L-hydroxyacyl-CoA. The hydroxyl group is oxidized by NAD^+ and a dehydrogenase to yield β-ketoacyl-CoA and NADH. The final step in the sequence involves a thiolytic cleavage to form acetyl-CoA and an acyl-CoA that is two carbons shorter than the initial substrate for β oxidation (Figure 13-7). This acyl-CoA can undergo another round of β oxidation to yield $FADH_2$, NADH, acetyl-CoA, and acyl-CoA. The enzymatic steps are repeated until in the last sequence of reactions, butyryl-CoA ($CH_3CH_2CH_2CO$—CoA) is degraded to two acetyl-CoAs.

The equations for the complete oxidation of palmitoyl-CoA are shown in Table 13-3. In Equation (1), the oxidation of palmitoyl-CoA by the enzymes of β oxidation is shown. Each of the products of Equation (1) is further oxidized by the respiratory chain (Equations 2 and 3) or by the tricarboxylic acid cycle and the respiratory chain (Equation 4). When these reactions (Equations 1 to 4) are added together, the result is Equation (5). Hence 1 molecule of palmitoyl-CoA can be oxidized to yield 16 CO_2 + 131 ATP + 146 H_2O and CoA. If the starting material were palmitic acid, its complete oxidation would yield 129 ATP + 16 CO_2 + 145 H_2O. The formation of the CoA derivative of the fatty acid requires the hydrolysis of two high-energy bonds, and one molecule of H_2O is consumed in the hydrolysis of the PP_i produced by the thiokinase reaction. Interestingly, oxidation of fatty acids can be used as a major source of H_2O, e.g., in the killer whale (which is actually a dolphin). This animal lives in the sea, but does not drink the seawater. Instead, the whale obtains a significant amount of its water from dietary fatty acids.

Table 13-3
Equations for the Complete Oxidation of Palmitoyl-CoA to CO_2 and H_2O

$$CH_3(CH_2)_{14}CO\text{—CoA} + 7\,FAD + 7\,H_2O + 7\,NAD^+ + 7\,CoA \longrightarrow 8\,CH_3COSCoA + 7\,FADH_2 + 7\,NADH + 7\,H^+ \quad (1)$$

$$7\,FADH_2 + 14\,P_i + 14\,ADP + 3\tfrac{1}{2}\,O_2 \longrightarrow 7\,FAD + 21\,H_2O + 14\,ATP$$

$$7\,NADH + 7\,H^+ + 21\,P_i + 21\,ADP + 3\tfrac{1}{2}\,O_2 \longrightarrow 7\,NAD^+ + 28\,H_2O + 21\,ATP \quad (3)$$

$$8\,Acetyl\text{-}CoA + 16\,O_2 + 96\,P_i + 96\,ADP \longrightarrow 8\,CoA + 104\,H_2O + 16\,CO_2 + 96\,ATP \quad (4)$$

$$CH_3(CH_2)_{14}CO\text{—CoA} + 131\,P_i + 131\,ADP + 23\,O_2 \longrightarrow 131\,ATP + 16\,CO_2 + 146\,H_2O + CoA \quad (5)$$

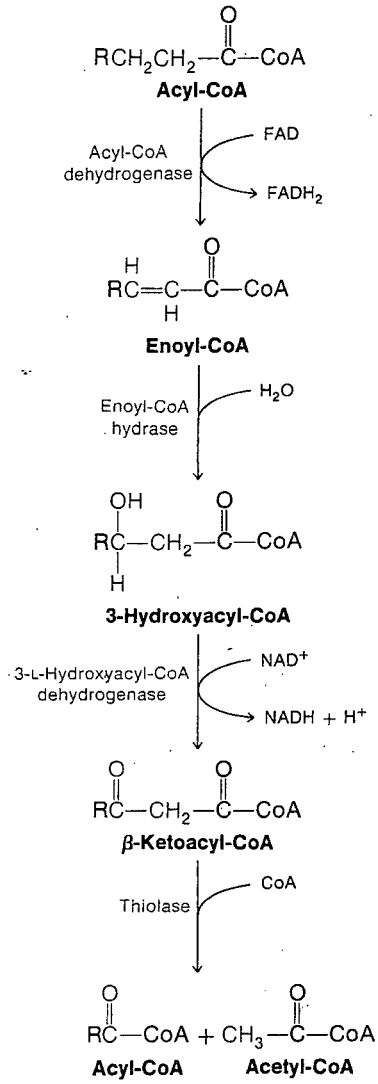


Figure 13-7
Scheme for β oxidation of fatty acids.

The yield of ATP from the oxidation of palmitic acid can be compared with that from glucose. Both are major sources of energy in our body. Since palmitate has 16 carbons and glucose has 6 carbons, the comparison should be made between 1 palmitate and $2\frac{2}{3}$ glucose molecules. As learned in Chapter 10, 36 ATPs are produced from the complete oxidation of glucose to CO_2 and H_2O. The yield from $2\frac{2}{3}$ glucose molecules is therefore 96 ATPs. Thus oxidation of palmitate yields an additional 33 ATPs. Therefore, palmitate is a more efficient molecule than glucose for storage of energy. Lipid is also less hydrated than carbohydrate and therefore takes up less space. These two factors are probably why fat is the major storage form of energy. The chemical reason for the difference in oxidative energy yield between glucose and palmitate is that palmitate is almost completely in the reduced state, whereas glucose is partially oxidized with six oxygens in the molecule.

Enzymology of β Oxidation

Three different flavoproteins have been found in mitochondria that catalyze the initial dehydrogenation of acyl-CoA. The enzymes show specificity for either short-, medium-, or long-chain acyl-CoAs and specifically yield the *trans*-α,β-enoyl-CoA. The molecular weight of all three enzymes is in the range of 140,000 to 200,000. A second dehydrogenase, called the electron-transferring flavoprotein (ETF), oxidizes the acyl-CoA dehydrogenases so that they can participate in another round of β oxidation.

Only a single enoyl hydrase with very broad specificity for the acyl group has been identified. The enzyme has a molecular weight of 210,000. Similarly, a single β-hydroxyacyl-CoA dehydrogenase appears to be involved in the oxidation of the L-hydroxy group. NAD^+ is the specific electron acceptor for this reaction. The last reaction in the fatty acid oxidation cycle is catalyzed by β-ketoacyl-CoA thiolase. A second thiolase has been identified that is specific for acetoacetyl-CoA.

In recent years, the enzymes of β oxidation also have been found in peroxisomes. At the present time, the role of these organelles in the metabolism of fatty acids has not been well-defined.

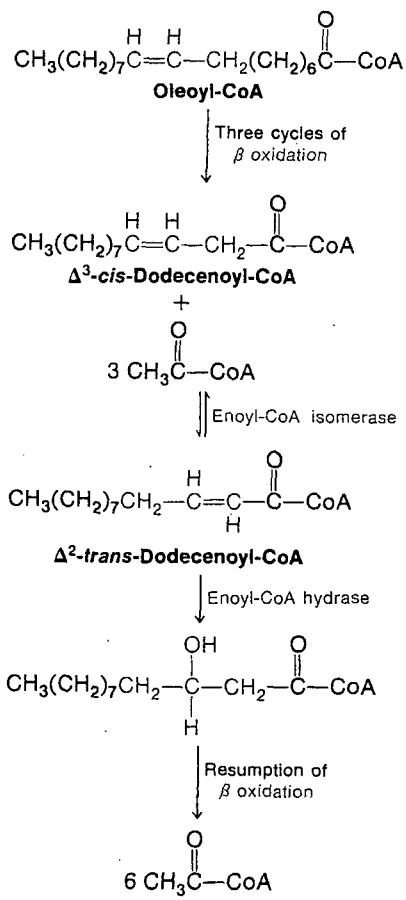


Figure 13-8
The β oxidation of oleoyl-CoA.

Oxidation of Unsaturated Fatty Acids

Unsaturated fatty acids are similarly degraded by β oxidation. As seen in Figure 13-8, oleic acid can be degraded in the same manner as stearic acid through the first three cycles of β oxidation. The resulting *cis*-3-dodecenoic acid ($12:1^{\Delta 3}$), however, is not a substrate for the acyl-CoA dehydrogenase. This step is bypassed by an isomerization of the double bond by *enoyl-CoA isomerase* to the *trans*-2-dodecenoyl-CoA, which is a normal substrate for enoyl-CoA hydratase, and the normal route for β oxidation resumes.

Polyunsaturated fatty acids also are degraded by β oxidation, but the process is aided by two additional enzymes, *enoyl-CoA isomerase* and *3-hydroxyacyl-CoA epimerase* (Figure 13-9). The degradation of linoleoyl-CoA begins, as with oleoyl-CoA, with three rounds of β oxidation and results in a *cis*-3-unsaturated fatty acid that is not a substrate for the acyl-CoA dehydrogenase. Isomerization of the double bond to the *trans*-2 posi-

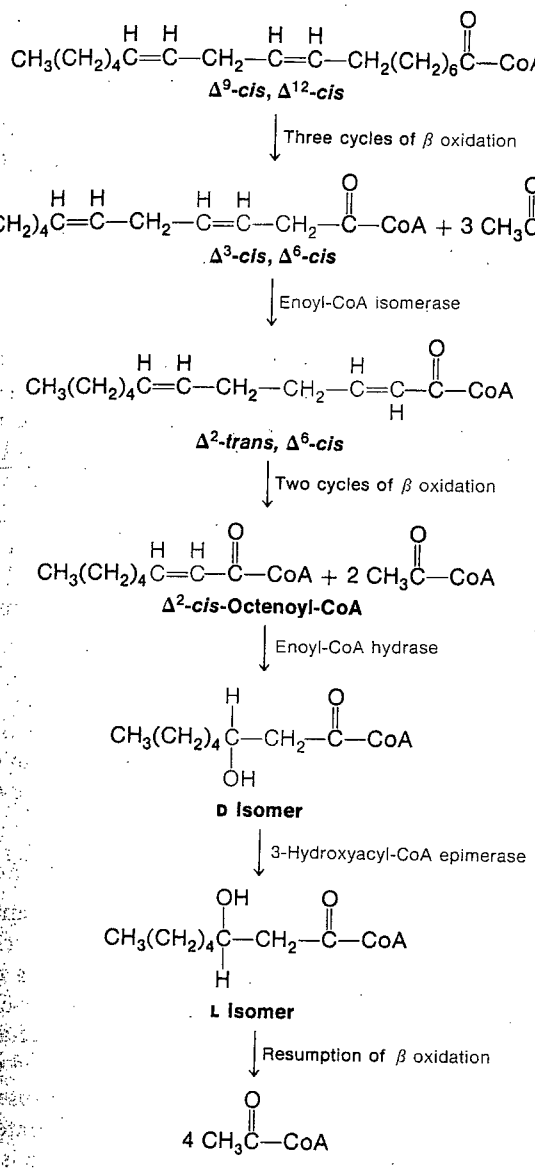


Figure 13-9
Pathway for the β oxidation of linoleoyl-CoA.

tion by enoyl-CoA isomerase allows for the resumption of two cycles of β oxidation. The product, *cis*-2-octenoyl-CoA, has the double bond in the correct position of the chain, but is cis rather than trans. The enoyl-CoA hydrase will hydrate this fatty acid. However, the product is in the D configuration and will not be further degraded by the 3-hydroxyacyl-CoA dehydrogenase, which is specific for the L configuration. This problem is resolved by the presence of 3-hydroxyacyl-CoA epimerase, which catalyzes the inversion of configuration at carbon 3. Subsequently, β oxidation can continue and completely degrade the rest of the acyl chain to 4 acetyl-CoAs.

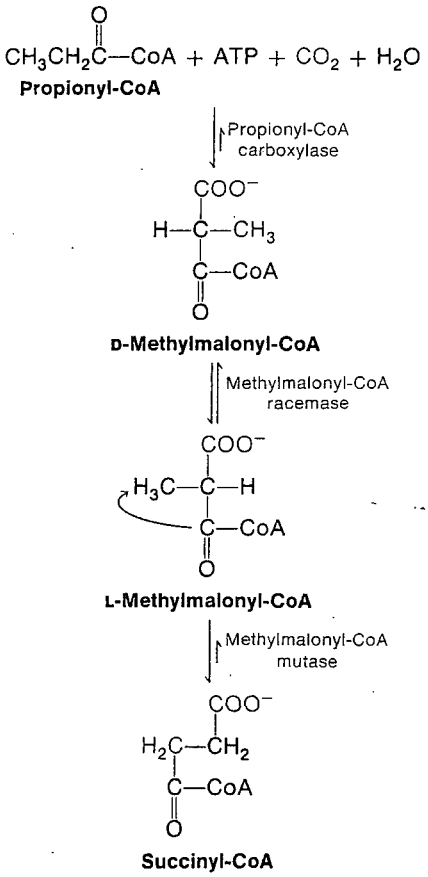
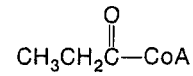


Figure 13-10
Conversion of propionyl-CoA to succinyl-CoA.

Oxidation of Fatty Acids with an Odd Number of Carbons

The amounts of fatty acids with an uneven number of carbons are very low in many mammalian tissues. However, in ruminant mammals, the oxidation of odd-chain fatty acids can account for as much as 25 percent of their energy requirements. Consequently, straight-chain fatty acids with 17 carbons will be oxidized by the normal β-oxidation sequence and give rise to 7 acetyl-CoAs and 1 propionyl-CoA:



This three-carbon acyl-CoA also is a product of degradation of the amino acids valine and isoleucine (see Chapter 23). The propionyl-CoA is converted to succinyl-CoA by three enzymatic steps, as indicated in Figure 13-10. The initial carboxylation is catalyzed by *propionyl-CoA carboxylase*, which utilizes biotin as a cofactor. In the second reaction, D-methylmalonyl-CoA is converted to its optical isomer, L-methylmalonyl-CoA by *methylmalonyl-CoA racemase*. The last step in the sequence involves an unusual migration of the carbonyl-CoA group to the methyl group in an exchange for hydrogen. The product, succinyl-CoA, can be metabolized in the tricarboxylic acid cycle.

Methylmalonyl-CoA mutase is a mammalian enzyme that requires cobalamin (see Chapter 6) for activity. The enzyme has been purified from sheep liver and human placenta. The human enzyme has a molecular weight of 145,000. The absence of this enzymatic activity in children with *congenital methylmalonicaciduria* results in death during childhood.

α Oxidation and Refsum's Disease

Although β oxidation is quantitatively the most significant pathway for catabolism of fatty acids, α oxidation of some fatty acids is essential to our well-being. In a normal diet, small amounts of *phytol*, a component of chlorophyll, are ingested. As shown in Figure 13-11, this long-chain alcohol is oxidized to *phytanic acid*, which is a more important dietary component present in ruminant fat and dairy products. The estimated daily intake of phytanic acid is somewhere between 50 and 100 mg. Because of the methyl substitution on carbon 3, phytanic acid is not a substrate for acyl-CoA dehydrogenase, the first enzyme in β oxidation. This step is circumvented by another mitochondrial enzyme that hydroxylates the α carbon of phytanic acid. The hydroxy intermediate is decarboxylated to yield *pristanic acid* and CO_2 (Figure 13-11). Pristanic acid is unsubstituted at carbon 3 and can be oxidized by acyl-CoA dehydrogenase and the normal enzymes of β oxidation to produce propionyl-CoA and an acyl-CoA. The latter can be degraded by four cycles of β oxidation, which yield, alternately, acetyl-CoA and propionyl-CoA. The final sequence of reactions produces acetyl-CoA and isobutyryl-CoA. The latter acyl-CoA can be converted into succinyl-CoA and subsequently metabolized by means of the tricarboxylic acid cycle.

Our current understanding of how humans metabolize phytol and phytanic acid came largely as a result of the studies of Daniel Steinberg and co-workers. The impetus for their experiments resulted from studies

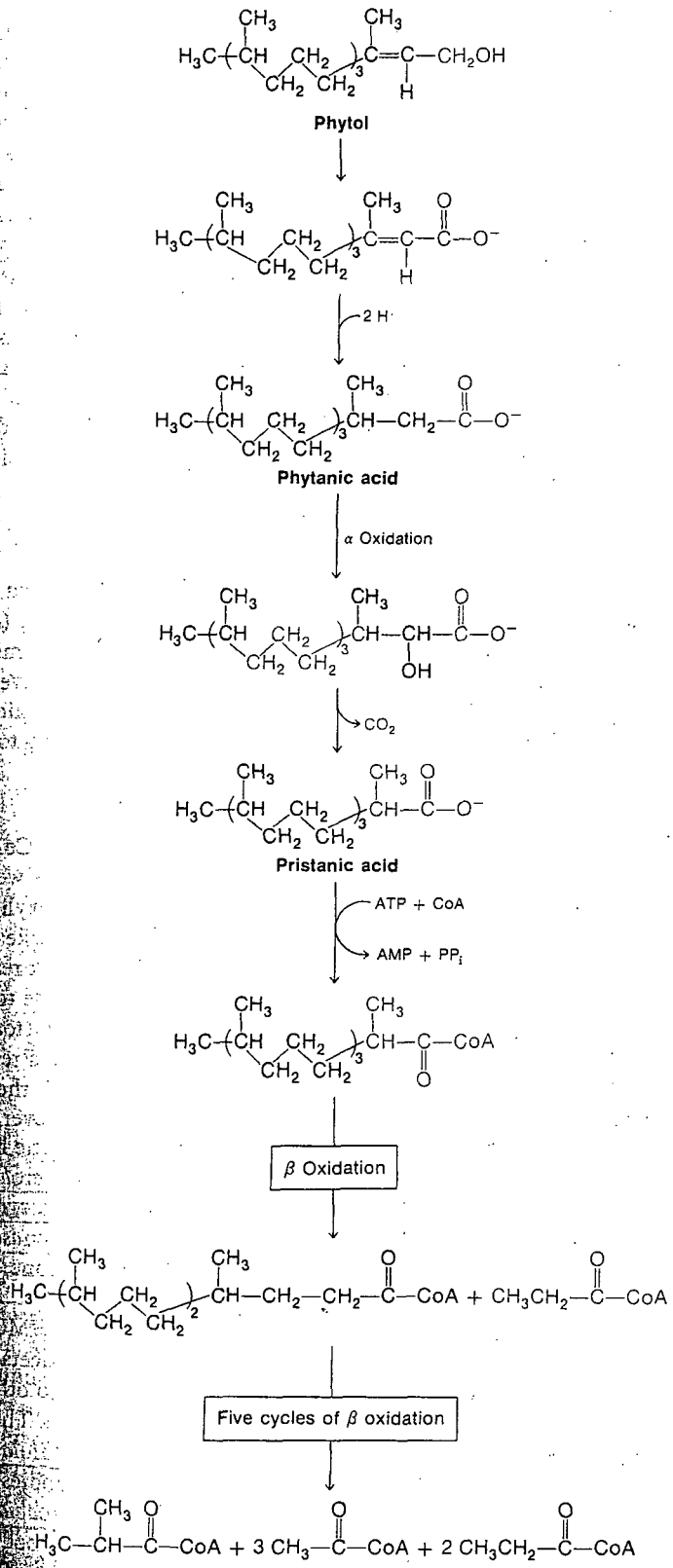


Figure 13-11
Scheme for oxidation of phytol.

on *Refsum's disease*, an inherited and extremely rare disorder characterized by numerous neurologic malfunctions—tremors, unsteady walking, constricted visual field, and poor night vision. The symptoms are probably due to an accumulation of phytanic acid throughout the nervous system. In these patients, 5 to 30 percent of the plasma fatty acids (20–100 mg/100 ml) and approximately 50 percent of the liver fatty acids was phytanic acid. In contrast, the normal amount of phytanic acid in plasma is below 1 percent (0.3 mg/100 ml). The disease is now known as *phytanic acid storage syndrome*. A series of biochemical studies has demonstrated that this fatty acid accumulates because of a deficiency in α oxidation of phytanic acid to pristanic acid. The metabolic defect is most likely in the α hydroxylation of phytanic acid, since people with the disorder are able to oxidize phytol to phytanic acid and pristanic acid to CO_2 and H_2O. Once these patients are identified, the symptoms of the disease can be diminished by a strict dietary regimen in which foods that contain phytanic acid are restricted.

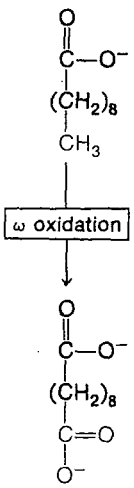


Figure 13-12
ω Oxidation of decanoic acid.

ω Oxidation

A minor pathway for the oxidation of fatty acids has been observed in rat liver microsomes. This involves oxidation of the terminal methyl (ω carbon) or adjacent methylene carbon of fatty acids by NADPH and molecular oxygen (Figure 13-12). This pathway is probably not quantitatively significant for the oxidation of long-chain fatty acids. However, ω oxidation may be important for the metabolism of short-chain fatty acids (C_6 to C_{10}).

Formation and Utilization of Ketone Bodies

Once fatty acids are degraded in the mitochondria, the acetyl-CoA can undergo a number of metabolic fates. Of central importance, as we have learned in Chapter 9, is utilization of acetyl-CoA by the tricarboxylic acid cycle. An alternate fate is the synthesis of *ketone bodies*, which takes place only in the mitochondria, as depicted in Figure 13-13. In the first reaction catalyzed by *acetoacetyl-CoA thiolase*, two acetyl-CoAs condense to form *acetoacetyl-CoA*. A third molecule of acetyl-CoA reacts with acetoacetyl-CoA to yield *β-hydroxy-β-methyl-glutaryl-CoA* (HMG-CoA) in a reaction catalyzed by *HMG-CoA synthase*. As we shall see in Chapter 15, the same two reactions are the first steps in cholesterol biosynthesis; however, these reactions take place only in the cytosol. In the formation of ketone bodies, the next reaction is catalyzed by *HMG-CoA lyase* and yields *acetoacetate* and acetyl-CoA. The acetoacetate can be reduced to *β-hydroxybutyrate* by the mitochondrial enzyme, *D-β-hydroxybutyrate dehydrogenase*. Although the acetoacetate also can be decarboxylated to form acetone, this is normally of minor importance.

Ketone body synthesis is primarily a liver function, since HMG-CoA synthase is present in large quantities only in this tissue. Acetoacetate and β-hydroxybutyrate diffuse into blood, where they are carried to other tissues and converted into acetyl-CoA, as described in Figure 13-14. The reactions catalyzed by *β-hydroxybutyrate dehydrogenase* and *thiolase* are common to both the synthesis and degradation of the ketone bodies. However, the second enzyme in the sequence (Figure 13-14), *β-oxoacid-CoA-transferase*, is present in all tissues but liver. Hence the ketone bodies are largely made in the liver and are metabolized to CO_2 and energy in nonhepatic tissues.

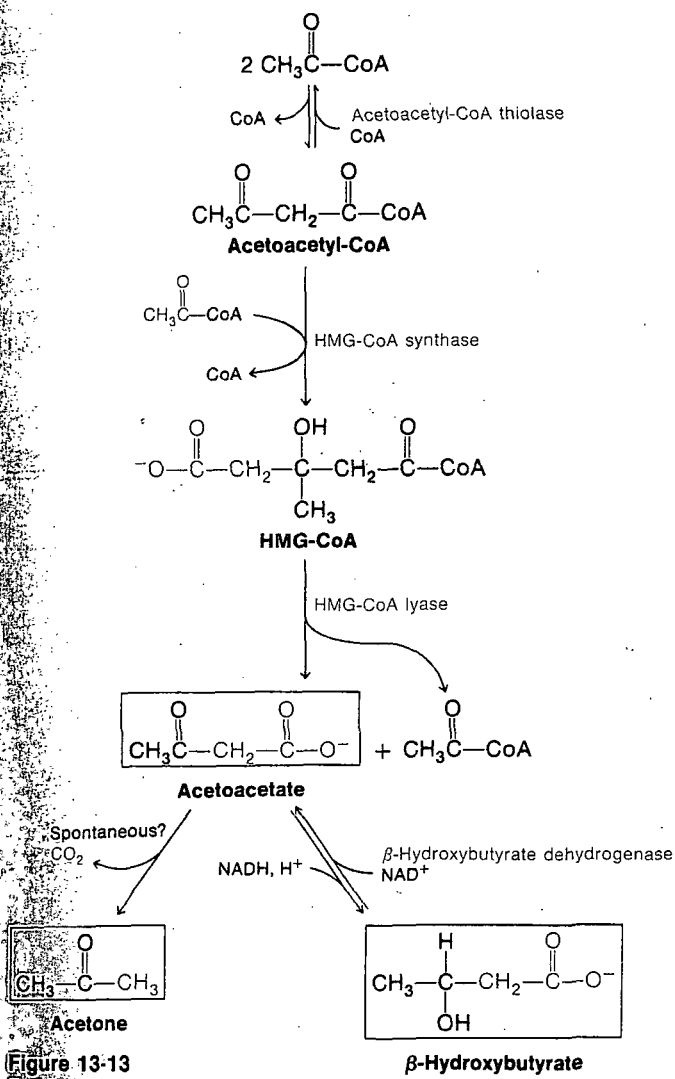


Figure 13-13
Synthesis of ketone bodies.

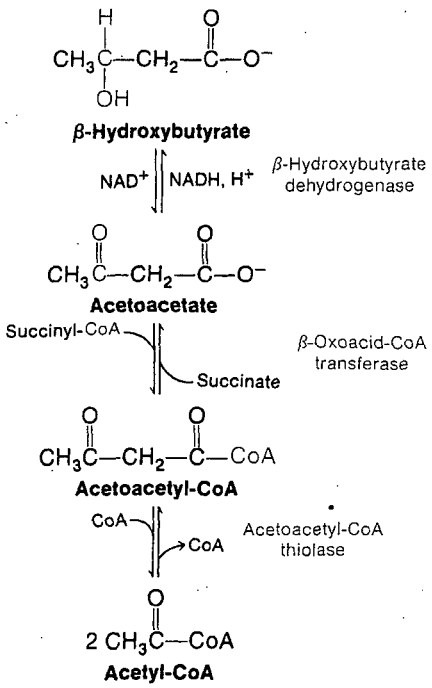


Figure 13-14
Metabolism of ketone bodies.

BIOSYNTHESIS OF FATTY ACIDS

Historical Developments

Once it was established that most fatty acids contained an even number of carbon atoms from C_4 to C_{20}, it was postulated by Rapier in 1907 that they were produced by condensation of a highly reactive two-carbon compound. After the introduction of isotopes in the late 1930s and early 1940s as a fundamental tool for the biochemist, experiments were performed that clearly implicated an acetate derivative as the two-carbon compound. This was demonstrated by David Rittenberg and Konrad Bloch in 1944 and 1945 by a series of experiments in which they fed mice acetate labeled with deuterium and ^{13}C ($C^2H_3{}^{13}COOH$) and found both isotopes incorporated into fatty acids. After Lynen discovered that "active acetate" was acetyl-CoA, a central role for this compound in fatty acid biosynthesis was demonstrated. Precisely how acetyl-CoA was converted into fatty acids eluded

workers until the late 1950s, when the involvement of *malonyl-CoA* was discovered. Subsequently, progress was rapid and the scheme for fatty acid biosynthesis as we know it today was elucidated.

Reactions of Saturated Fatty Acid Biosynthesis

All cells, from *E. coli* to liver, appear to synthesize fatty acids by the same chemical reactions. However, there are fascinating variations in the structure and organization of the enzymes that catalyze the reactions. Best understood is the pathway for the biosynthesis of palmitic acid as its *acyl carrier protein(ACP)-thioester* in *E. coli*, as shown in Figure 13-15. This

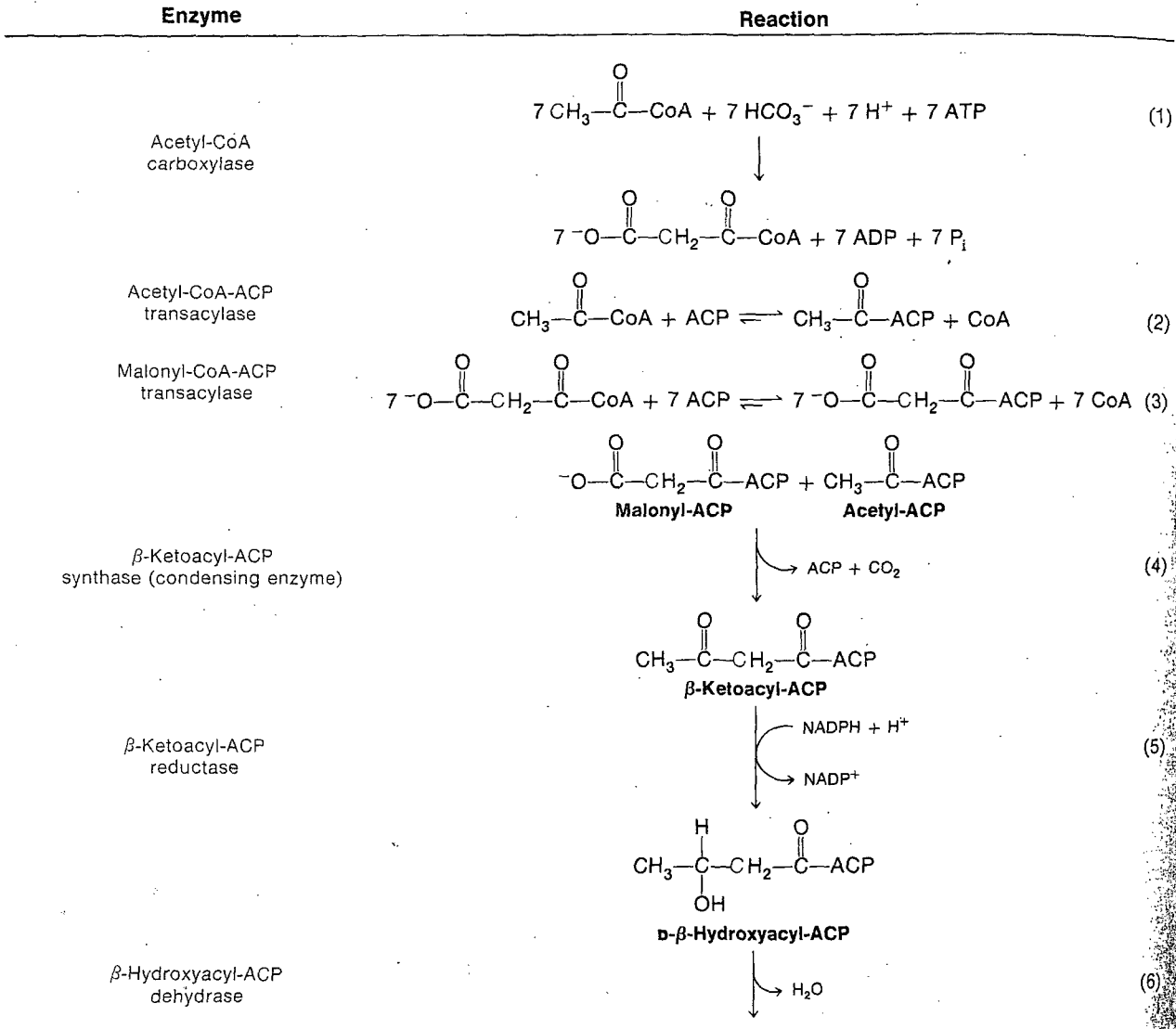


Figure 13-15
Scheme for biosynthesis of palmitoyl-ACP in *E. coli*.

protein has a molecular weight of 8847 in *E. coli* and functions as the carrier of the acyl residue during fatty acid biosynthesis. There are approximately 15×10^6 molecules of *acyl carrier protein* per cell, the most abundant protein found in *E. coli*. ACP, like CoA, contains one *phosphopantetheine* per molecule of protein (Figure 13-16). It is the sulfhydryl group of the phosphopantetheine to which acetyl and acyl groups are esterified.

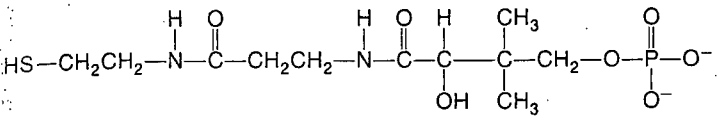


Figure 13-16
Structure of phosphopantetheine.

Enzyme	Reaction
(1) Enoyl-ACP reductase	$CH_3-CH=CH-C(=O)-ACP$ (*α,β-trans*-Enoyl-ACP) $\xrightarrow{NADPH + H^+,\ -NADP^+}$ $CH_3-CH_2-CH_2-C(=O)-ACP$ (Butyryl-ACP) (7)
(2) (3) CoA	(Repeat reactions 4–7) $\xrightarrow{-O-C(=O)-CH_2-C(=O)-ACP + 2\,NADPH + 2\,H^+,\ -ACP + 2\,NADP^+ + H_2O + CO_2}$ $CH_3CH_2CH_2CH_2CH_2-C(=O)-ACP$
(4) (5)	(Recycle reactions 4–7 five times) $\xrightarrow{5\,-O-C(=O)-CH_2-C(=O)-ACP + 10\,NADPH + 10\,H^+,\ -5\,ACP + 10\,NADP^+ + 5\,H_2O + 5\,CO_2}$ $CH_3CH_2(CH_2CH_2)_6CH_2-C(=O)-ACP$ (Palmitoyl-ACP)

(6)

$$8\ CH_3-C(=O)-CoA + ACP + 7\ HCO_3^- + 7\ ATP + 14\ NADPH + 21\ H^+ \longrightarrow CH_3(CH_2)_{14}-C(=O)-ACP + 8\ CoA + 14\ NADP^+ + 7\ ADP + 7\ P_i + 7\ CO_2 + 7\ H_2O \quad (8)$$

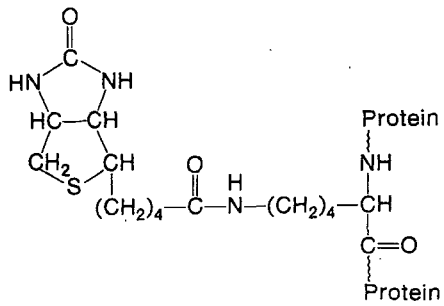


Figure 13-17
Structure of biotin linked to BCCP.

The first reaction in the biosynthetic sequence is the carboxylation of acetyl-CoA to malonyl-CoA (Reaction 1, Figure 13-15). Subsequently, acetyl-CoA and malonyl-CoA are transacylated to yield the corresponding ACP-thioesters (Reactions 2 and 3) that condense in an essentially irreversible reaction to form β-ketoacyl-ACP (Reaction 4). This β-ketoacyl-ACP is reduced with NADPH to D-β-hydroxyacyl-ACP (Reaction 5), which is stereospecifically dehydrated to yield the α,β-*trans*-enoyl-ACP (Reaction 6). The L-β-hydroxyacyl-ACP is not a substrate for this dehydration. The double bond is reduced with NADPH (Reaction 7), and the resulting butyryl-ACP serves as a substrate for another condensation with malonyl-ACP (Reaction 4.) This reaction sequence (Reactions 4–7) continues to recycle until palmitoyl-ACP is produced after a total of 43 reactions have taken place. The palmitoyl-ACP is either transacylated to the CoA derivative or used directly in phospholipid biosynthesis, as discussed in Chapter 14.

Acetyl-CoA Carboxylase

The initial reaction of fatty acid biosynthesis in all cells is catalyzed by *acetyl-CoA carboxylase* (Reaction 1, Figure 13-15). The activity of this enzyme appears to play an important role in the control of fatty acid biosynthesis in mammals, yeast, and probably most organisms and tissues. The enzyme contains the vitamin biotin covalently linked by means of the ε amino group of a lysine in the protein (Figure 13-17). The carboxyl group is initially transferred to the biotin moiety by an ATP-requiring step. In a second reaction, the carboxyl is transferred to the methyl carbon of acetyl-CoA.

Of the many acetyl-CoA carboxylases known, the enzyme from *E. coli* is perhaps best understood. The enzyme consists of three protein components: *biotin carboxyl carrier protein* (BCCP) (M_r = 22,500), *biotin carboxylase* (M_r = 98,000; two subunits of 49,000), and *carboxyltransferase* (M_r = 130,000). The carboxyltransferase component has an A_2B_2 structure, and the molecular weights of the two types of subunits are 35,000 and 30,000. The reaction sequence (Figure 13-18) involves an initial carboxylation of BCCP, catalyzed by biotin carboxylase. Subsequently, the carboxyltransferase transfers the CO_2 from BCCP to acetyl-CoA. The probable mechanism for the carboxyltransferase reaction is shown in Figure 13-19.

Acetyl-CoA carboxylase is found in the cytosol of animal livers and has been purified from rat and chicken liver. The rat liver enzyme has a molecular weight of 460,000 and is composed of two subunits (M_r = 230,000). There is one biotin per subunit. It is possible that the three functional parts of acetyl-CoA carboxylase occur as a single multifunctional polypeptide in rat liver. Although this form of the enzyme (*protomer*) is essentially inac-

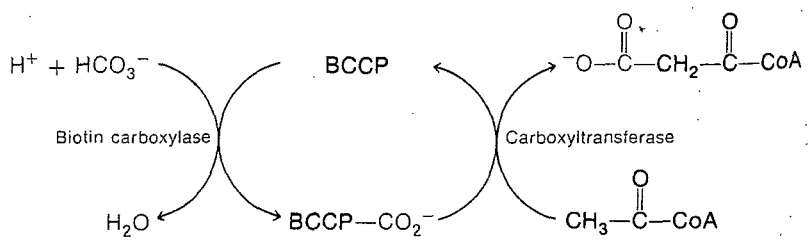


Figure 13-18
Reactions catalyzed by acetyl-CoA carboxylase from *E. coli*.

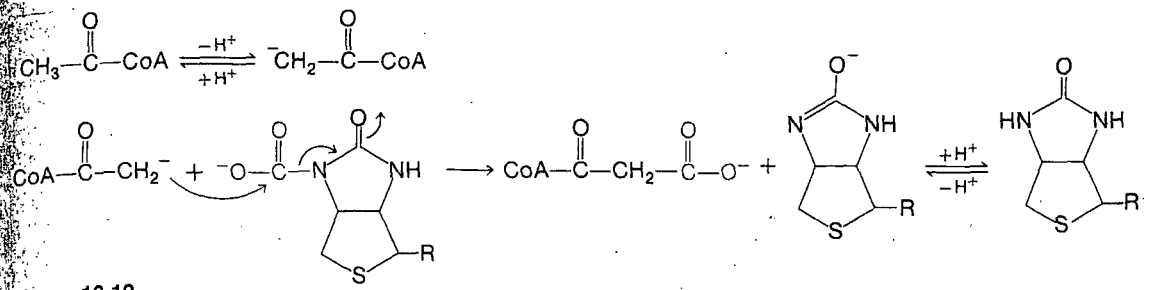


Figure 13-19
Carbanion mechanism for the carboxylation of acetyl-CoA.

tive, incubation with citrate results in polymerization to an active form with a molecular weight of between 4 and 8 million (Figure 13-20). The enzyme is deactivated and depolymerized when incubated with malonyl-CoA or palmitoyl-CoA. The significance of these modulators on the rate of fatty acid biosynthesis will be discussed in the section entitled Control of Fatty Acid Metabolism. Citrate will not cause polymerization–depolymerization of the *E. coli* enzyme.

The Fatty Acid Synthase of *E. coli*

Nature has developed a splendid diversity in the organization of the enzymes that catalyze Reactions 2 to 7 of Figure 13-15. Although composed of at least six enzymatic activities, the enzyme is usually referred to in the singular as *fatty acid synthase*. The activity of fatty acid synthase is assayed by the incorporation of [2-^{14}C]malonyl-CoA or [^{3}H]acetyl-CoA into fatty acid, as shown in Figure 13-21. This scheme illustrates a common principle often utilized in the assay of lipid biosynthetic enzymes. The radioactive substrate is a water-soluble molecule that can easily be separated from the lipid product by extraction of the reaction mixture with an organic solvent, such as petroleum ether. When the fatty acid synthase is highly purified, it also can be assayed by a spectrophotometric method in which the oxidation of NADPH is followed. As $NADP^+$ is formed, there is a decrease in the absorbance at 340 nm.

The fatty acid synthase from *E. coli* exists as a group of enzymatic activities that can be separated from one another by conventional methods of purification. Table 13-4 summarizes some of the main properties of the *E. coli* enzymes.

The ACP from *E. coli* has been purified to homogeneity, and the amino acid sequence has been determined (Figure 13-22). The phosphopantetheine is linked to Ser-36. ACP and various analogs have been chemically synthesized by the solid-phase method developed by Bruce Merrifield. This has facilitated studies on the structural requirement of the ACP for biological activity. The three C-terminal amino acids could be removed with no loss of biological activity. Although removal of 16 amino acids from the C-terminal resulted in a decrease in activity, the polypeptide still functioned at a low rate. In contrast, as the N-terminal amino acids were removed from ACP, there was a progressive loss of activity. The protein without six N-terminal amino acids was inactive. The details of how the acyl-ACP interacts with the fatty acid biosynthetic enzymes are unknown.

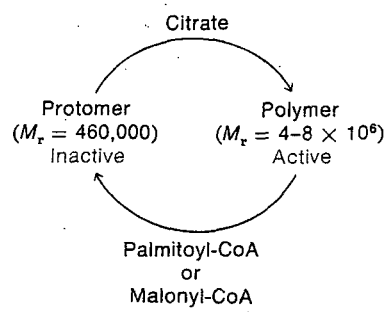


Figure 13-20
Activation of acetyl-CoA carboxylase from rat liver.

Step 1. Prepare incubation mixture, which contains:
0.3 m*M* NADPH
0.1 *M* phosphate buffer, pH 6.8
5 μM mercaptoethanol
3 μM EDTA
50 μM malonyl-CoA
12 μM [^{3}H]Acetyl-CoA (specific radioactivity = 2.0×10^6 dpm/μmol).
Distilled H_2O, so final volume will be 1 ml.

Step 2. Equilibrate mixture for 5 min at 37°C in a shaking water bath.

Step 3. Add enzyme (e.g., 2 mg protein), mix thoroughly, and incubate at 37°C for 5 min.

Step 4. Stop the reaction by the addition of 0.1 ml 18% perchloric acid.

Step 5. Add 1 ml ethanol and 2 ml petroleum ether, mix thoroughly, and allow the phases to separate.

Step 6. Transfer the ether layer to a liquid scintillation vial. Extract the aqueous phase two more times with petroleum ether.

Step 7. Evaporate the combined petroleum ether extracts, add liquid scintillation fluid, and determine the radioactivity by liquid scintillation spectrophotometry.

Step 8. Calculate the specific activity of the enzyme. The specific activity is calculated by dividing the dpm incorporated into the fatty acid by the specific radioactivity of the acetyl-CoA, the time of the incubation, and the milligrams of protein added to the assay.

Example:

$$\text{Specific activity} = \frac{\text{dpm in fatty acid}}{\text{specific radioactivity of acetyl-CoA} \cdot \text{min} \cdot \text{mg protein}}$$

$$= \frac{50{,}000 \text{ dpm}}{2 \times 10^6 \text{ dpm}/\mu\text{mol} \cdot 5 \text{ min} \cdot 2 \text{ mg protein}}$$

$$= 2.5 \times 10^{-3} \, \mu\text{mol fatty acid formed/min} \cdot \text{mg protein}$$

Figure 13-21
An assay for fatty acid synthase from rat liver.

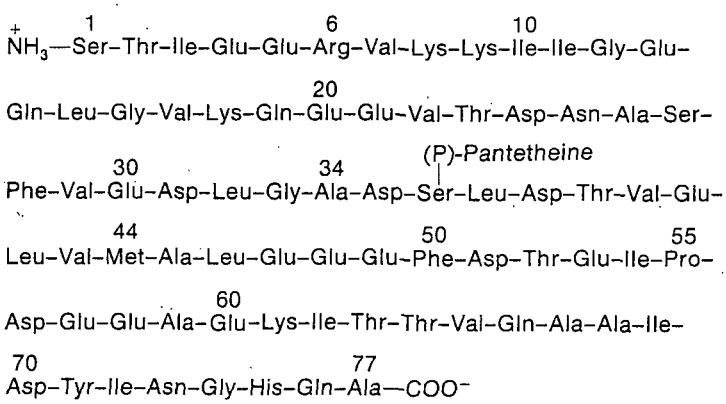


Figure 13-22
The amino acid sequence of ACP from *E. coli*.

Table 13-4
The Enzymes of Fatty Acid Synthase from *E. coli*

Enzyme	M_r	Subunits	Specificity	Miscellaneous
Acetyl-CoA-ACP transacylase	–	–	Acetyl-CoA 100% Butyryl-CoA 10% Hexanoyl-CoA 4.5%	Acetyl-CoA + enz. $\rightleftharpoons$ acetyl-enz. + CoA-SH Acetyl-enz. + ACP $\rightleftharpoons$ acetyl-*S*-ACP + enz.
Malonyl-CoA-ACP transacylase	36,700	None	Acetyl-CoA is a competitive inhibitor; $K_i = 115\ \mu M$	Malonyl-CoA + enz. $\rightleftharpoons$ malonyl-enz. + CoA-SH Malonyl-enz. + ACP-SH $\rightleftharpoons$ malonyl-ACP + enz. Malonate is esterified to serine on the enzyme
β-Ketoacyl-ACP synthase I	80,000	$2 \times 40{,}000$, apparently identical	Active with C_2–C_{14} ACP, but inactive with C_{16} ACP; inactive with CoA derivatives	Acetyl-*S*-ACP + enz.-SH $\rightleftharpoons$ acetyl-*S*-enz. + ACP-SH Acetyl-*S*-enz. + malonyl-*S*-ACP $\rightleftharpoons$ acetoacetyl-*S*-ACP + CO_2 + enz.-SH
β-Ketoacyl-ACP synthase II	88,000	$2 \times 44{,}000$	Active with 16:$1^{\Delta 9}$-ACP as substrate	
β-Ketoacyl-ACP reductase	–	–	Specific for NADPH; active with C_4–C_{16} β-ketoacyl-ACP	The product is the D configuration
β-Hydroxyacyl-ACP dehydrase	–	–	Specific for D isomer; inactive with L-β-hydroxyacyl-ACP; active with C_4–C_{16} derivatives; lowest activity with C_{10} substrate; inactive with CoA derivatives	The product is trans
Enoyl-ACP reductase	–	–	There may be two enzymes, one specific for NADH and one for NADPH	

Of the enzymes of fatty acid synthase, only *malonyl-CoA: ACP transacylase* and *β-ketoacyl-ACP synthases I and II* have been purified to homogeneity. The other four enzymes have been partially purified and some properties have been determined. None of the enzymes has been sequenced nor have the three-dimensional structures been determined. Moreover, it is not clear whether the enzymes exist separately or as an aggregate in the intact organism. Clearly, we have only a primitive understanding of how these enzymes synthesize fatty acids.

The Fatty Acid Synthase of *Mycobacterium smegmatis*

Most bacteria have a fatty acid synthase that resembles the *E. coli* enzyme. However, Konrad Bloch and co-workers discovered that the phylogenetically more advanced bacteria such as *mycobacteria* have high-molecular-weight fatty acid synthases with multifunctional polypeptides. Bloch's studies on the enzyme from *Mycobacterium smegmatis* have revealed an enzyme with many unusual features. The enzyme has a molecular weight

A possible structural arrangement of the yeast fatty acid synthase is shown in Figure 13-23. The actual arrangement of the enzymatic activities and the ACP on the two peptides has not been established. It is also unclear how the acyl residue on ACP can serve as a substrate for the various enzymatic reactions. An understanding of the structure of the fatty acid synthase remains a difficult, but fascinating problem.

The fatty acid synthases from rat liver, pigeon liver, and many other tissues are found in the cytosol and have been purified. The liver enzyme has a molecular weight of around 500,000, with two apparently identical polypeptides (M_r = 240,000). Each giant peptide contains one molecule of phosphopantetheine. It is thought that each multifunctional polypeptide contains the ACP region, all the activities for fatty acid synthesis, and a palmitoyl esterase that hydrolyzes the palmitoyl residue from the enzyme. Recent evidence suggests that in the presence of CoA, the palmitoyl moiety is transferred to CoA. The three-dimensional arrangement of the enzymatic activities in these multifunctional polypeptides is a problem of current interest.

The apparently rapid evolution of the multienzyme complexes in bacteria and the retention in most eukaryotes suggest that this arrangement of the fatty acid synthase may have conferred a selective advantage. Clearly, the multifunctional polypeptides avoid the accumulation of intermediates and provide equivalent stoichiometry for each of the component enzyme activities.

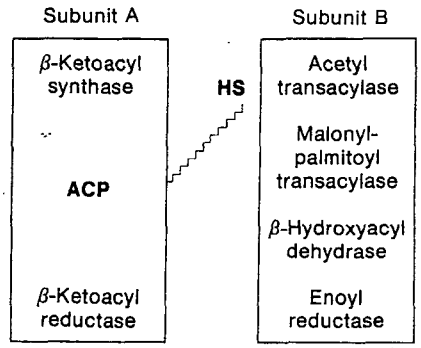


Figure 13-23
Possible structural arrangement of A-B subunit of yeast fatty acid synthase.

Biosynthesis of Monounsaturated Fatty Acids

Two chemically distinct mechanisms exist for the introduction of the cis double bond into monounsaturated fatty acids: the *anaerobic pathway*, as typified in *E. coli*; and the *aerobic pathway*, found in eukaryotes.

The anaerobic pathway has been studied most intensely in the laboratory of Konrad Bloch at Harvard University. As the name "anaerobic" implies, the double bond of the fatty acid is inserted in the absence of oxygen. Biosynthesis of these fatty acids follows the pathway described previously for saturated fatty acids in *E. coli* until the intermediate *β-hydroxydecanoyl-ACP* is reached (Figure 13-24). At this point, there is an apparent competition between *β-hydroxyacyl-ACP dehydrase*, which forms an α,β-trans double bond, and *β-hydroxydecanoylthioester dehydrase*, which forms a β,γ-cis double bond. This β,γ unsaturated acyl-ACP is subsequently elongated by the fatty acid synthase to yield palmitoleoyl-ACP ($16:1^{\Delta 9}$). The conversion of this compound to the major unsaturated fatty acid of *E. coli*, *cis*-vaccenic acid ($18:1^{\Delta 11}$) appears to involve β-ketoacyl-ACP synthase II, which shows in vitro a preference for palmitoleoyl-ACP as a substrate (see Table 13-4). The subsequent conversion of β-keto-*cis*-vaccenyl ACP to *cis*-vaccenic acid is catalyzed by the usual enzymes of fatty acid biosynthesis.

β-Hydroxydecanoylthioester dehydrase has been purified. It has a molecular weight of 36,000 with two apparently identical subunits. The enzyme is highly specific for the dehydration of C_{10}-β-hydroxyacyl-ACP and has no activity with the C_8 or C_{12} homologs.

In contrast to the anaerobic pathway, the aerobic pathway in eukaryotic cells introduces double bonds *after* the saturated fatty acid has been synthesized. In rat liver and other eukaryotic cells, an enzyme complex

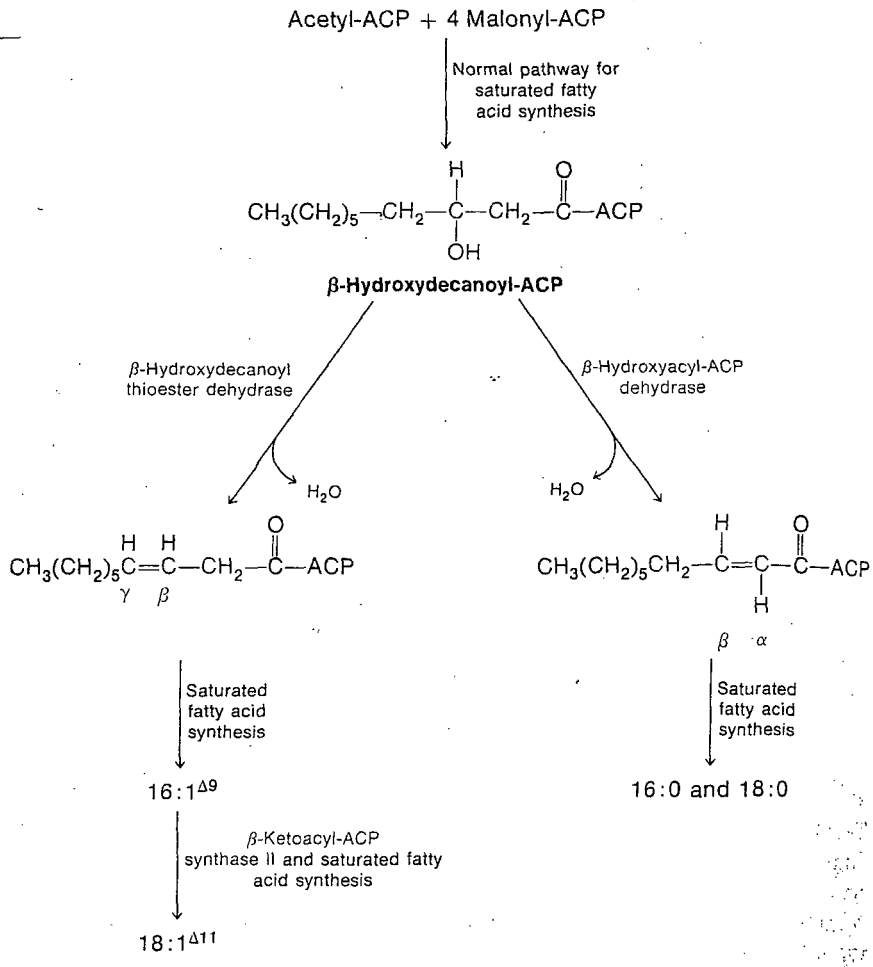


Figure 13-24
Anaerobic pathway for biosynthesis of monounsaturated fatty acids.

associated with the endoplasmic reticulum desaturates stearoyl-CoA to oleoyl-CoA. This reaction requires NADH and O_2 and results in the remarkable formation of a double bond in the middle of an acyl chain with no activating groups nearby. Although many elegant experiments have been performed, the chemical mechanism for desaturation of long-chain acyl-CoAs remains unclear.

Desaturation requires the cooperative action of two enzymes and cytochrome b_5. A scheme for this set of reactions is shown in Figure 13-25. *Cytochrome b_5 reductase* (M_r = 43,000) is a flavoprotein that transfers electrons from NADH by means of flavin (F) to *cytochrome b_5*, a heme-containing protein (M_r = 16,700) in which Fe^{3+} is reduced to Fe^{2+}. Both cytochrome b_5 and the reductase are *amphipathic proteins* with a hydrophobic peptide tail that anchors the protein into the membrane and a hydrophilic portion that is outside the membrane surface (see Chapter 16 for details about membrane-bound proteins). *Stearoyl-CoA desaturase* utilizes two electrons from cytochrome b_5 coupled with an atom of oxygen to form a cis double bond in the Δ^9 position of stearoyl-CoA. The desaturase

(M_r = 53,000) has 62 percent nonpolar amino acids, which is probably the main reason it is tightly embedded in the membrane. There is also one atom of non-heme iron per molecule of enzyme.

Biosynthesis of Polyunsaturated Fatty Acids

E. coli does not have polyunsaturated fatty acids, whereas eukaryotes produce a large variety of polyunsaturated fatty acids. Animals cannot desaturate beyond the Δ^9 position of an acyl chain, whereas plants have the enzymes to desaturate at positions Δ^{12} and Δ^{15}. Thus animals have a dietary requirement for linoleic and linolenic acids. However, enzyme complexes occur in the endoplasmic reticulum of animal cells that desaturate at Δ^5 if there is a double bond at the Δ^8 position or Δ^6 if there is a double bond at the Δ^9 position. These enzymes are different from Δ^9-desaturase, but they also appear to utilize cytochrome b_5 reductase and cytochrome b_5.

The major polyunsaturates of animals are either derived from diet or from desaturation and elongation of $18:2^{\Delta 9,12}$ or $18:3^{\Delta 9,12,15}$. A scheme for the synthesis of arachidonic acid ($20:4^{\Delta 5,8,11,14}$) from linoleic acid is shown in Figure 13-26, which illustrates the principle by which polyunsaturated fatty acids are made in animals. The elongation step is catalyzed by a series of membrane-bound enzymes that are present in the endoplasmic reticulum. These enzymes use malonyl-CoA as the donor for the C_2 unit, and the chemical mechanism seems to be similar to that described earlier for fatty acid synthesis (Figure 13-15). The liver enzymes also will elongate other polyunsaturated fatty acyl-CoAs. In addition, microsomal enzymes will elongate C_{16} and C_{18}-acyl-CoAs to produce the C_{22}- and C_{24}-CoAs characteristic of sphingolipids (see Chapter 14). The latter elongation enzymes are most active in brain tissue during the synthesis of myelin.

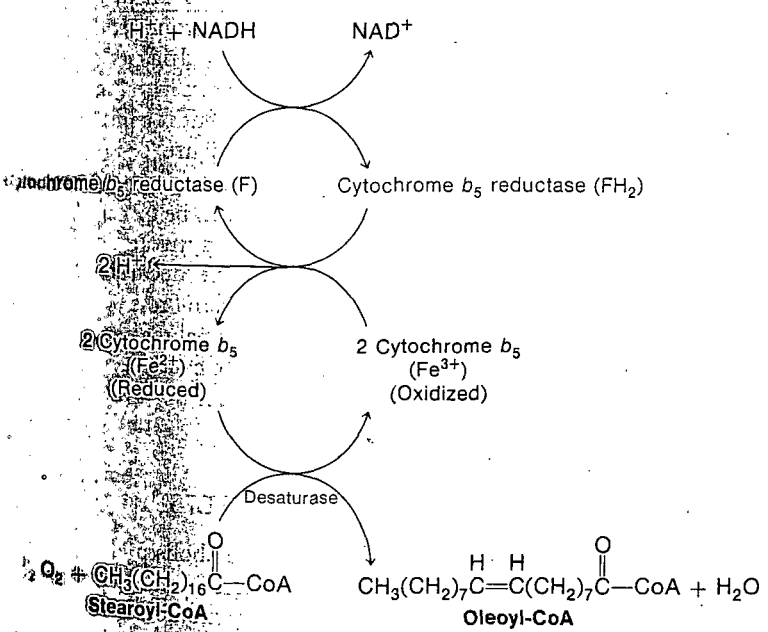


Figure 13-25
Formation of oleoyl-CoA in eukaryotes. (In some instances, NADPH has been found to be the electron donor for desaturation.)

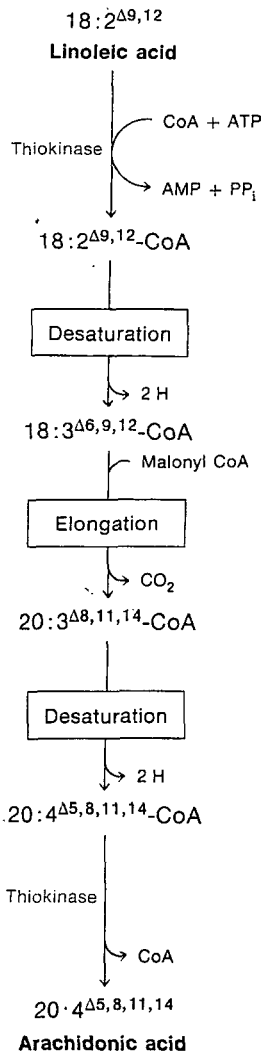


Figure 13-26
Synthesis by mammalian tissues of arachidonic acid from linoleic acid.

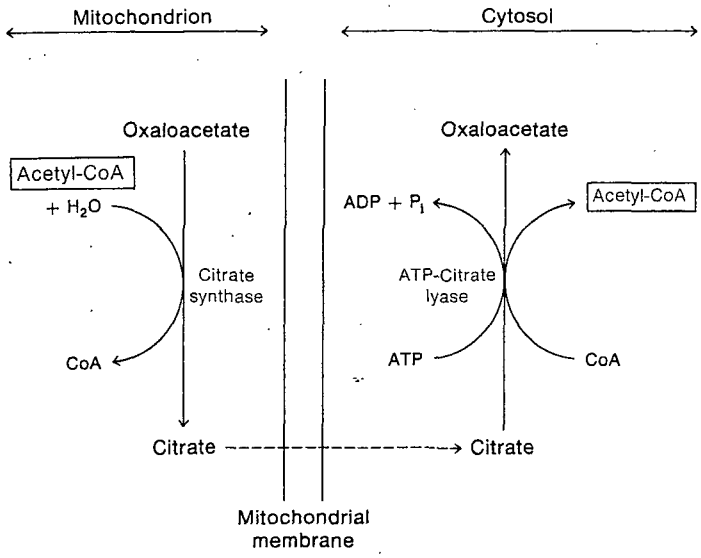


Figure 13-27
Transfer of acetyl-CoA from a mitochondrion to the cytosol.

Metabolism Related to Fatty Acid Synthesis

During periods of excess food supply, an animal will store energy as fat. Thus excess carbohydrate will be converted to pyruvate, which will in turn be degraded to acetyl-CoA by pyruvate dehydrogenase in the mitochondria. (Acetyl-CoA is also produced from the degradation of certain amino acids.) Acetyl-CoA is not readily transported into the cytosol, where the enzymes of fatty acid synthesis occur. However, in the mitochondria, *citrate synthase* will convert acetyl-CoA and oxaloacetate into citrate, which will cross the mitochondrial membranes. In the cytosol, *ATP-citrate lyase* catalyzes the formation of acetyl-CoA and oxaloacetate from citrate (Figure 13-27). Thus the acetyl-CoA generated in the mitochondria can be used for fatty acid synthesis in the cytosol. The oxaloacetate produced in the cytosol cannot cross the mitochondrial membranes, but it can be returned to the mitochondria after conversion to malate (Figure 13-28). Alternatively, the malate can be oxidatively decarboxylated to pyruvate by *malic enzyme* in the cytosol (Figure 13-28). The pyruvate can be transported back to the mitochondria and converted to acetyl-CoA by pyruvate dehydrogenase or to oxaloacetate by pyruvate carboxylase. The decarboxylation of malate by malic enzyme appears to play an important role in fatty acid synthesis by generation of NADPH, which is an essential reducing agent for fatty acid synthesis. Other reactions, notably the one catalyzed by *glucose-6-P dehydrogenase,* also appear to be important sources of NADPH for fatty acid synthesis.

CONTROL OF FATTY ACID METABOLISM

As the student will now appreciate, the rates of metabolism are controlled by a variety of different mechanisms, and fatty acid metabolism is no exception. The major mechanisms for control of any metabolic pathway are given in Table 13-5. In this section we will discuss these possible control mechanisms as applied to fatty acid synthesis in animals and, where appro-

Table 13-5
General Mechanisms for Control of a Metabolic Pathway

1. Change in the concentration of active enzyme:
   - Affected by enzyme synthesis and degradation.
   - Affected by covalent modifications.
2. Change in the concentration and availability of substrates and products.
3. Change in the supply of covalent and noncovalent cofactors.
4. Change in activators and inhibitors: active site and allosteric.

priate, comment on their physiological importance. Fatty acid synthesis in *E. coli* seems to be linked closely with phospholipid synthesis; thus control of both these biosynthetic pathways will be discussed together in Chapter 14.

A first consideration in the control of a metabolic pathway is: which enzyme catalyzes the rate-limiting reaction? The slowest reaction in a metabolic sequence is the most appropriate point for control, because this "bottleneck" determines the overall flux of the pathway. There would be little point in altering the rate of a reaction if it were in any case faster than the slowest step in the pathway. For example, *there is evidence that acetyl-CoA carboxylase catalyzes the rate-limiting reaction for fatty acid synthesis in a number of tissues and cells* (e.g., liver). *Thus acetyl-CoA carboxylase would be the most suitable enzyme for control of fatty acid synthesis.*

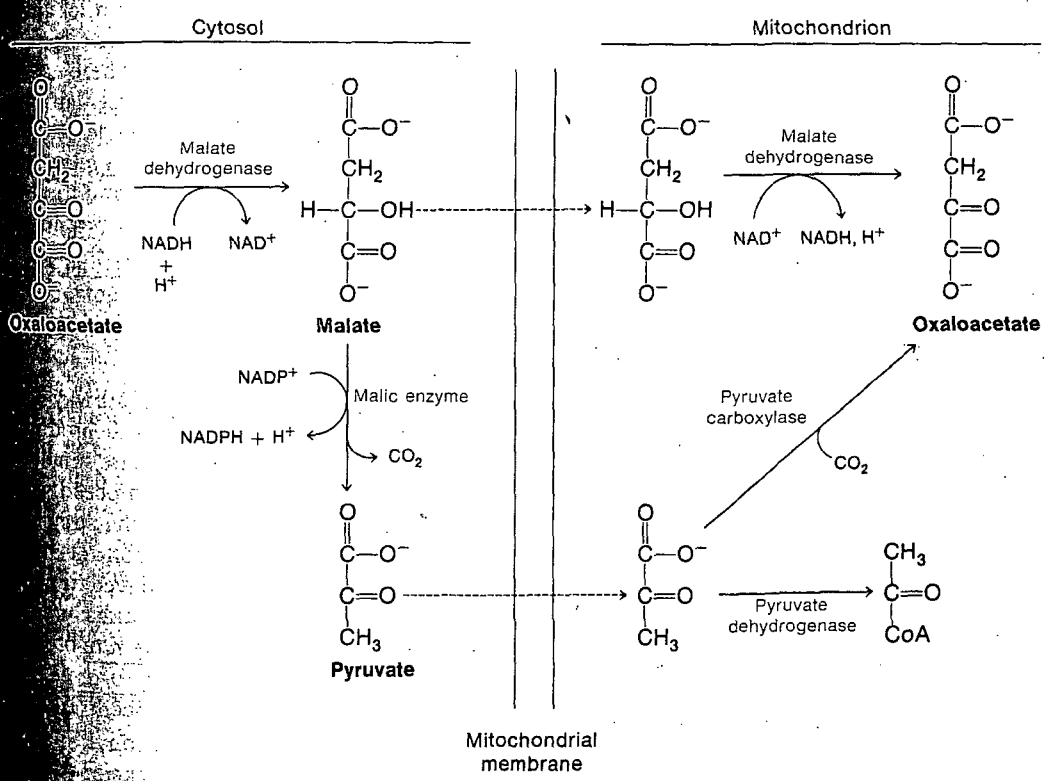


Figure 13-28
Oxaloacetate metabolism in the cytosol and mitochondrion.

Nevertheless, there is at least one exception, because acetyl-CoA carboxylase does not catalyze the rate-limiting reaction for fatty acid synthesis in *M. smegmatis*.

Concentration of Acetyl-CoA Carboxylase and Fatty Acid Synthase

One major mechanism for control of fatty acid synthesis is a change in the concentration of active enzyme(s) available for catalysis. Genetic factors, stage of development, hormones, and energy supply are important elements that dictate the amount of acetyl-CoA carboxylase, fatty acid synthase, malic enzyme, and other enzymes related to fatty acid synthesis. For example, the concentrations of fatty acid synthase and acetyl-CoA carboxylase in rat liver are reduced fourfold to fivefold after fasting. When the rat is allowed to eat again, the concentrations of fatty acid synthase and acetyl-CoA carboxylase rise dramatically. If the rat is fed a fat-free diet, the concentration of fatty acid synthase is 14-fold higher than in a rat maintained on a normal rat chow. *Current evidence strongly suggests that the levels of these enzymes are governed by the rate of enzyme synthesis, not degradation*. Thus a decreased level of acetyl-CoA carboxylase occurs because less enzyme is made, while the enzyme is degraded at the normal rate. Similarly, higher concentrations of enzyme are due to an increased rate of synthesis. It appears that the change in the rate of enzyme synthesis is due to a fluctuation in the supply of mRNA, which in turn is governed by the rate of transcription of DNA. A question of current interest is how this transcription of mRNA is controlled.

The alteration in enzyme levels is an *adaptive*, or long-term, change, since the response occurs over a period of hours or days. A faster response can be mediated by activation or inactivation of an enzyme. There is evidence that the activity of acetyl-CoA carboxylase in rat liver is regulated by a covalent phosphorylation-dephosphorylation mechanism. The less active form of the enzyme is phosphorylated. *Insulin*, which stimulates fatty acid synthesis, causes dephosphorylation of acetyl-CoA carboxylase and thereby activation of the enzyme. *Glucagon* causes the reverse effect by promoting the synthesis of cyclic AMP, which activates a protein kinase that phosphorylates and inactivates acetyl-CoA carboxylase.

Substrates and Products

A second general mechanism for control of a metabolic pathway is a change in the concentration and availability of substrates and products. Clearly, a cell will not synthesize fatty acids without an adequate supply of cytosolic acetyl-CoA and malonyl-CoA, nor will β oxidation proceed without sufficient acyl-CoA in the mitochondria. Regulation of the supply of acetyl-CoA would not be a particularly good candidate for control of the rate of fatty acid synthesis, since it is a central metabolite involved in many different reactions (for example, synthesis of isoprene compounds and sterols; see Chapter 15). *Considerable evidence suggests that the rate of fatty acid synthesis and degradation is determined by the supply of malonyl-CoA*. Thus the rate of fatty acid synthesis increases as the concentration of malonyl-CoA increases in the cytosol. At the same time, malonyl-CoA inhibits carnitine acyltransferase I and thereby reduces the transfer of acyl-CoA into the mitochondria for β oxidation.

The major product of fatty acid synthesis, palmitoyl-CoA, is a potent inhibitor of many enzymes, including acetyl-CoA carboxylase, fatty acid synthase, citrate synthase, and glucose-6-P dehydrogenase. It appears that the carboxylase is the most sensitive to palmitoyl-CoA and is inhibited by dissociation into its inactive protomer. Inhibition of this activity will cause the decreased synthesis of malonyl-CoA, which will in turn limit fatty acid synthesis. This is not the case in *M. smegmatis*, where the fatty acid synthase is more sensitive to palmitoyl-CoA than the carboxylase. As discussed earlier, a unique regulatory mechanism has evolved for control of fatty acid synthesis in this organism.

Supply of Cofactors

A third mechanism for control of fatty acid metabolism is a change in the rate of supply of such cofactors as biotin, NADPH, pantetheine, and carnitine. If an animal becomes deficient in biotin, clearly this will affect the activity of biotin-dependent carboxylases. Thus a biotin-deficient animal may not synthesize fatty acids under conditions that normally favor this process because of a deficiency of *holo*-acetyl-CoA carboxylase. Similarly, a limited supply of pantetheine in the diet would adversely affect the supply of CoA and *holo*-fatty acid synthase and would eventually have dramatic effects on fatty acid metabolism. However, these possibilities for control probably operate only in extreme circumstances.

The level of carnitine also can be important in the control of fatty acid oxidation. In addition, if sufficient adenosine diphosphate (ADP) were not present, the oxidation of NADH and $FADH_2$ would not proceed, and therefore, the rate of β oxidation would be decreased.

Activators and Inhibitors

A fourth category for control of metabolism is alteration in the concentration and availability of activators and inhibitors. As already mentioned, citrate plays a key role as an allosteric activator of acetyl-CoA carboxylase (Figure 13-20), as well as in the transport of acetate across mitochondrial membranes. *There is much evidence, particularly in chicken liver, for a central role for citrate in modulation of the rate of fatty acid synthesis.* The cytoplasmic level of citrate is decreased by glucagon, which causes a rapid inhibition of fatty acid synthesis in chicken liver cells that are maintained in culture. The effect of glucagon is mediated by cAMP, which somehow causes a decrease in cytoplasmic citrate. It is postulated that the lower citrate levels result in a depolymerization and, as a result, inactivation of the acetyl-CoA carboxylase.

Methylmalonyl-CoA can inhibit fatty acid synthesis as an active-site competitor with malonyl-CoA. In vitamin B_{12} deficiency, methylmalonyl-CoA accumulates because of a decreased activity of methylmalonyl-CoA mutase (Figure 13-10). However, the B_{12}-deficient rat, at least, compensates for possible inhibition of fatty acid synthesis by methylmalonyl-CoA with an increase in the concentration of acetyl-CoA carboxylase.

Conclusion

The rate of fatty acid synthesis and degradation is governed by a complex set of interactions among DNA, RNA, proteins, carbohydrates, and lipids. Although desirable, it would be an extremely complex task to set forth a

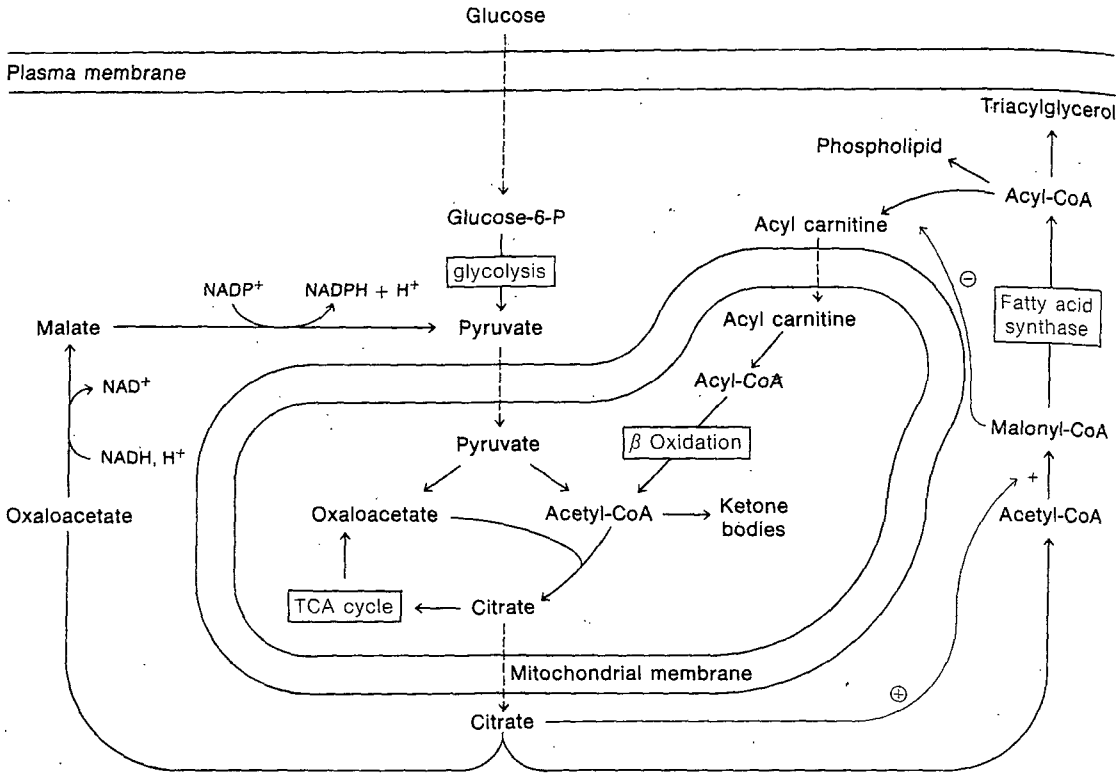


Figure 13-29
Conversion of glucose to lipid in liver cells.

unifying and comprehensive scheme for control of fatty acid metabolism. However, a scheme for the major metabolic events in the conversion of glucose to fatty acid is outlined in Figure 13-29. By glycolysis, plus the action of three mitochondrial enzymes, glucose is converted into citrate. The citrate migrates into the cytosol, where it is metabolized to acetyl-CoA, which is carboxylated to form malonyl-CoA. These two latter substrates are used for fatty acid synthesis. The fatty acyl-CoAs are used for triglyceride and phospholipid synthesis and are diverted from β oxidation by inhibition of carnitine acyltransferase I by malonyl-CoA. In glucose deprivation, the levels of citrate and malonyl-CoA in the cytosol would fall and the rate of fatty acid synthesis would decrease. At the same time, fatty acids would be mobilized from triglyceride stores and would be transported into the mitochondria for β oxidation.

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Quantitative Structure–Activity Relationships for Skin Irritation and Corrosivity of Neutral and Electrophilic Organic Chemicals

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Abstract—Quantitative structure–activity relationships (QSARs) have been derived by relating skin irritation and corrosivity data of neutral and electrophilic organic chemicals to their log(octanol/water partition coefficient) (logP), molecular volume, dipole moment and 1/molecular weight. Datasets were analysed using stepwise regression, discriminant and principal components analysis. Discriminant analysis between irritant and non-irritant neutral and electrophilic organic chemicals using the above parameters, which broadly model skin permeability (logP and molecular volume), 'reactivity' (dipole moment) and 1/molecular weight to compensate for the fact that skin irritation/corrosivity testing is carried out using a fixed mass or volume of chemical, was found to discriminate well for only 73.1% of the dataset (67.3% cross-validated). The poor discrimination at the irritant/non-irritant classification boundary is attributed largely to biological variability. Stepwise regression analysis of the Primary Irritation Index (PII) for the same dataset showed a poor correlation ($r^2 = 0.422$; cross-validated $r^2 = 0.201$) with a positive dependence on logP and dipole moment and a negative dependence on molecular volume; 1/molecular weight was not a significant variable. While this QSAR for PII has little value as a predictive model, mainly because of the large biological variability evident in PII values, it is useful in confirming the putative model for skin irritation. Discriminant analysis using logP, molecular volume and dipole moment, was able to discriminate reasonably well (92.9% well-classified; 92.9% cross-validated) between corrosive and non-corrosive electrophiles. A plot of the first two principal components of the same parameters showed a clear demarcation between corrosive and non-corrosive electrophiles. In contrast to the QSARs for skin irritation, increasing skin corrosivity was found to correlate with decreasing molecular volume, with increasing dipole moment, and with *decreasing* logP. The predominant parameter in determining the skin corrosivity of electrophilic organic chemicals appears to be the molar dose at which they are tested; this arises because skin corrosivity testing is conducted using a fixed mass or volume of chemical. A stepwise approach to the skin corrosivity/irritation classification of neutral and electrophilic organic chemicals is outlined. The derived QSARs should be useful for the prediction of the skin corrosivity potential of new or untested electrophiles. (Non-electrophilic neutral organic chemicals, as a category, do not generally appear to be corrosive.) Discrimination between some non-irritant and irritant neutral and electrophilic organic chemicals using these techniques is also possible. For a large number of chemicals whose irritation potentials lie in a fairly broad band around the irritant/non-irritant classification boundary, no firm prediction of classification is possible. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The principles of QSARs are based on the premise that the properties of a chemical are implicit in its molecular structure. It therefore follows that if the mechanism responsible for the activity of a group of chemicals can be elucidated and relevant parameters measured or calculated, then in principle, a structure–activity relationship can be established.

A substance which is classified as 'corrosive' is one that causes irreversible destruction of skin tissue when applied to rabbit skin for a period of up to 4 hr as defined in the EC Annex V method (EEC, 1984). In earlier work, the relationship between the skin corrosivity of organic acids, bases and phenols and parameters modelling skin permeability and cytotoxicity was demonstrated (Barratt, 1995a). It was hypothesized that the chemical must first penetrate into the skin; having done this, cell death would occur beneath the stratum corneum if the dose and cytotoxicity of the chemical was sufficient. The parameters used to model the mechanism were the same as those used to model percutaneous absorption, that is, logP, molecular volume and melting point (Barratt, 1995b), together with a measure of cytotoxicity (pK_a).

In this paper, the same principles are applied to a study of the skin irritation and corrosivity potentials of neutral and electrophilic organic chemicals; however, only two of the three parameters used in the previous study (Barratt, 1995a) are used to model the skin permeability part of the mechanism. Melting point, a parameter used to compute aqueous solubility when linked with logP (Suzuki, 1991), is

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not used as a parameter in the current study since almost all of the chemicals in the study are in the liquid state at ambient temperature. The parameter used to model the 'reactivity' component is dipole moment; this parameter has been used successfully as the reactivity parameter in a QSAR study of the eye irritation potential of neutral organic chemicals (Barratt, 1995c). The use of dipole moment as a reactivity parameter for the eye irritation QSAR was based on the knowledge that some low molecular weight neutral molecules can alter the electrical resistance (and thus the ion permeability) of phospholipid membranes (Cherry *et al.*, 1970; Johnson and Bangham, 1969). Changes in electrical resistance (and hence ion permeability) in the membranes of the eye are postulated to lead to cytotoxicity and result in irritation. Diethyl ether, chloroform, *n*-butanol (Johnson and Bangham, 1969) and aliphatic alcohols with chain lengths up to eight carbons (Cherry *et al.*, 1970)—all chemicals with a significant dipole moment—were all found to decrease the electrical resistance of liposomes, whereas hexane, cyclohexane and benzene (chemicals with small dipole moments) produced no significant changes in resistance (Cherry *et al.*, 1970). The validity of dipole moment as a reactivity parameter in this study is discussed below.

An additional parameter is used in this study—1/molecular weight—to compensate for the fact that the EC Annex V method for skin irritation/corrosivity is conducted using a fixed mass or volume of chemical rather than a molar dose.

MATERIALS AND METHODS

Chemical structures were constructed using Sybyl 6.1 software (Tripos Associates, Bracknell, UK). After energy minimization and calculation of logP values using the CHEMICALC system (Suzuki and Kudo, 1990), the structures were imported into the TSAR spreadsheet (Oxford Molecular Ltd, Sandford-on-Thames, UK) where molecular volumes and dipole moments were calculated. Datasets were analysed using stepwise regression, discriminant and principal components analysis. All cross validation was carried out using a fixed deletion pattern of three subsets of data; three trials were carried out with each subset omitted once.

Most of the data used in this study are taken from the ECETOC Skin Irritation and Corrosion Chemicals Data Bank (ECETOC, 1995). They consist of Primary Irritation Index (PII) and EC classification data derived as follows.

Erythema and oedema grades were scaled following OECD Guideline 404 (OECD, 1993). PII values were calculated by ECETOC according to the formula

$$\mathrm{PII} = \frac{\Sigma \text{ erythema at } 24/48/72 \text{ hr} + \Sigma \text{ oedema at } 24/48/72 \text{ hr}}{3 \times \text{no. of animals}}$$

'Irritant' or 'Corrosive' classifications were generated by the author from the individual erythema and oedema scores in the data bank, using EC guidelines (EEC, 1993). In order to facilitate identification of the original data, the names of the chemicals used in this paper are the same as those in the ECETOC data bank (1995), rather than their IUPAC equivalents.

As only three of the electrophiles in the ECETOC data bank were classified as corrosive, six additional corrosive electrophiles and two corrosive pro-electrophiles (chemicals that are converted to electrophiles in the skin) were added from Annex I of the EC Dangerous Substances Directive (EEC, 1993) for the purposes of distinguishing corrosives from non-corrosives.

RESULTS AND DISCUSSION

Skin irritation

The dataset of 52 neutral and electrophilic organic chemicals, their PII values, classifications (irritant/non-irritant) together with logP, molecular volume, dipole moment and 1/molecular weight is shown in Table 1. Discriminant analysis of the dataset (Table 2) showed 38 (35 cross-validated) out of the 52 chemicals to be well classified on the basis of the four parameters with irritancy to skin correlating with increasing values of logP, dipole moment and 1/molecular weight and with decreasing molecular volume.

Stepwise regression analysis of PII for the same chemicals *v.* logP, molecular volume, dipole moment and 1/molecular weight resulted in the expression shown below, in which 1/molecular weight was not a significant variable:

$$\mathrm{PII} = 1.047\ \mathrm{logP} - 0.0244\ \mathrm{MV} + 0.888\ \mathrm{dipole} + 0.353$$

$n = 52$; $r^2 = 0.422$; cross-validated $r^2 = 0.201$; $s = 1.376$; $F = 11.70$; t(logP) = 5.25; t(MV) = 3.20; t(dipole) = 4.83.

A plot of the actual values of PII *v.* those predicted from this relationship is shown in Fig. 1. The actual irritant/non-irritant classifications of the 52 chemicals, together with those chemicals misclassified by discriminant analysis, are also indicated on the plot.

The predictive value of the regression plot in Fig. 1 is clearly very low. The use of regression as a technique for the analysis of a dependent variable such as PII values might be criticized on the grounds that PII is not strictly a continuous variable but is made up of small but discrete steps; however, because there are a large number of steps for PII (about 100 in the range 0 to 7.17), the approximation to a continuous variable is a reasonable one. This regression equation is valuable in two ways. First, it is useful in supporting the putative model for skin irritation, that is, a positive dependence on logP and a negative dependence on molecular volume as is also

the case for skin permeability (Barratt, 1995b), together with a positive dependence on dipole moment, the reactivity parameter. Secondly, it is clear from Fig. 1 that the 17 chemicals that are misclassified by discriminant analysis form a band with predicted values of PII lying between 2.07 and 3.48. This range of predicted PII values represents the range of uncertainty for the discriminant analysis. Chemicals predicted to be non-irritant by discriminant analysis and with predicted PII values below 2.07 have a high probability of being non-irritant, while those predicted to be irritant by discriminant analysis and with predicted PII values above 3.48 have a high probability of being irritant. No firm predictions of skin irritation classification can be made for chemicals with predicted PII values between 2.07 and 3.48.

Examination of the ECETOC dataset reveals variability at a number of different levels. Comparison of PII scores from different tests carried out on reputedly identical chemicals demonstrates that differences in excess of 1.5 units are possible with differences of 1 unit commonplace, for example the four tests on cyclamen aldehyde gave PII values of 3.42, 4.17, 4.83 and 5.11. Examination of individual animal scores also show wide variations in response for the same chemical applied at the same dose to different animals. It is common within the same test,

Table 1. Dataset of 52 organic chemicals

Chemical	PII	Class.	logP	MV (Å³)	Dipole moment (Debyes)	1/mol. wt (Daltons⁻¹)
1. 1,5-Hexadiene	0.0	U	2.634	77.49	0.0	0.01217
2. Methyl trimethyl acetate	0.0	U	1.419	98.73	2.032	0.00861
3. 1-Bromo-4-fluorobenzene	0.330	U	3.359	92.06	0.604	0.00571
4. Ethyl trimethyl acetate	0.50	U	1.904	112.4	2.020	0.00768
5. Isopropanol	0.780	U	0.154	55.18	1.470	0.01664
6. *n*-Butyl propionate	1.080	U	2.005	110.4	2.057	0.00768
7. *cis*-Cyclooctene	1.890	U	3.824	102.8	0.212	0.00907
8. Methyl caproate	2.780	U	2.056	110.9	1.963	0.00768
9. 1,9-Decadiene	3.0	I†	4.778	133.4	0.0	0.00723
10. Methyl laurate	3.890	I	5.272	188.3	1.962	0.00466
11. Methyl palmitate	4.560	I	7.416	239.9	1.962	0.00370
12. Benzyl acetate	1.195	U	1.574	116.7	2.009	0.00666
13. Benzyl benzoate	1.580	U†	3.680	164.1	2.265	0.00471
14. 1-Decanol	3.330	I†	4.096	151.7	1.389	0.00632
15. 2-Phenylethanol	1.570	U	1.420	100.4	1.526	0.00818
16. Dipropylene glycol	0.170	U	−1.003	109.1	3.103	0.00745
17. Diacetyl	0.630	U	−1.086	68.11	4.666	0.01161
18. Ethyl triglycol methacrylate	0.220	U	1.102	190.3	3.658	0.00406
19. 2-Ethoxyethyl methacrylate	1.560	U	1.268	122.7	3.656	0.00632
20. 2,4-Decadienal	4.790	I	3.532	128.7	4.763	0.00657
21. 3,7-Dimethyl-2,6-nonadienal	3.750	I	3.892	145.2	3.816	0.00601
22. *cis*-Jasmone	2.580	I	2.327	125.0	3.890	0.00666
23. Ethyl tiglate	1.170	U	2.245	103.5	2.130	0.00780
24. Cinnamaldehyde	3.710	I	1.894	97.60	4.266	0.00757
25. Isobutanal	0.130	U	0.363	64.90	2.779	0.01387
26. Cyclamen aldehyde	4.380	I	3.172	143.2	2.713	0.00567
27. 2-Ethylhexanal	3.880	I	2.507	118.6	2.805	0.00780
28. Heptanal	5.0	I	2.495	104.5	2.816	0.00876
29. 3-Methylbutanal	2.830	U	1.348	78.40	2.827	0.01161
30. Nonanal	3.460	I	3.567	130.7	2.815	0.00703
31. 2,5-Methylene-6-propyl-3-cyclohexylcarboxaldehyde	2.420	U†	3.403	145.1	2.708	0.00567
32. 2-Phenylpropanal	2.920	I†	1.606	103.7	2.881	0.00745
33. *p-iso*-Propylphenylacetaldehyde	2.290	U†	3.160	129.9	2.834	0.00616
34. Hexylcinnamic aldehyde	3.520	I	5.099	181.2	3.645	0.00462
35. Hydroxycitronellal	1.020	U	1.960	153.6	3.922	0.00580
36. 4-Methylthiobenzaldehyde	0.890	U†	2.196	108.6	2.989	0.00657
37. *p-tert*-Butyldihydrocinnamaldehyde	2.420	U†	3.996	157.7	2.802	0.00525
38. 2,4-Dimethyl-3-cyclohexene-1-carboxaldehyde	3.210	I†	2.218	120.2	2.850	0.00723
39. Salicylaldehyde	2.540	U†	1.876	88.85	3.425	0.00819
40. 1-Bromo-4-chlorobutane	0.0	U	2.572	98.40	0.058	0.00583
41. 1,6-Dibromohexane	0.890	U	3.776	128.4	0.003	0.00410
42. 2-Bromopropane	1.440	U†	2.652	70.13	1.973	0.00813
43. 1,3-Dibromopropane	1.890	I†	2.168	90.02	1.869	0.00495
44. 2-Bromobutane	2.440	U†	3.188	81.24	1.951	0.00730
45. 1-Bromohexane	4.0	I	3.724	108.0	1.940	0.00606
46. 1-Bromopentane	4.440	I	3.188	94.78	1.953	0.00602
47. Dihydromercenol	2.710	U	3.067	141.4	1.456	0.00648
48. Allyl bromide	7.170	I† (C)	1.726	65.15	1.801	0.00827
49. Fluorobenzene	0.110	U #	2.450	65.94	1.445	0.01040
50. Methacrolein	4.110	I (C)#	0.585	56.30	3.607	0.01427
51. Ethyl thioethyl methacrylate	0.560	U†	1.948	133.4	4.021	0.00574
52. 2,4-Hexadienal	7.080	I (C)	1.517	75.83	4.744	0.01040

I = Irritant U = Unclassified I(C) = Chemicals classified as corrosive
†Chemicals misclassified by discriminant analysis: #misclassified by cross-validated discriminant analysis.

Table 2. Discriminant analysis of 52 organic chemicals

Step no.	Variable entering	Total fraction well classified	Well classified fraction per class: U	I
1	MV	0.615	0.645	0.571
2	Dipole moment	0.615	0.613	0.619
3	logP	0.712	0.742	0.667
4	1/mol. wt	0.731	0.742	0.714
Cross-validation confidence estimates:		0.673	0.677	0.667

U = unclassified I = irritant

Number of individuals predicted to belong to each class by final model (cross-validated prediction in parenthesis)

	Predicted class: U	I	(Total)
True class:			
U	23 (21)	8 (10)	31 (31)
I	6 (7)	15 (14)	21 (21)
Total	29 (28)	23 (24)	

Composition of discriminant axes:

Variable	Axis Z1
logP	0.728
MV	−0.012
Dipole moment	0.586
1/mol. wt	0.355

for one or more animals to exhibit a response which is clearly 'irritant', while others may show little effect. The variabilities of this response pattern feed through into the EC classification system (EEC, 1993) which defines two different scoring systems depending on whether the test has been carried out using three or more animals. One consequence of there being two scoring systems is that a chemical with a skin irritation potential which is on the irritant/non-irritant threshold, is less likely to classify as a skin irritant if it is tested in more than three animals; a second consequence which is more important in the context of this work is the introduction of an additional source of variability into the biological data.

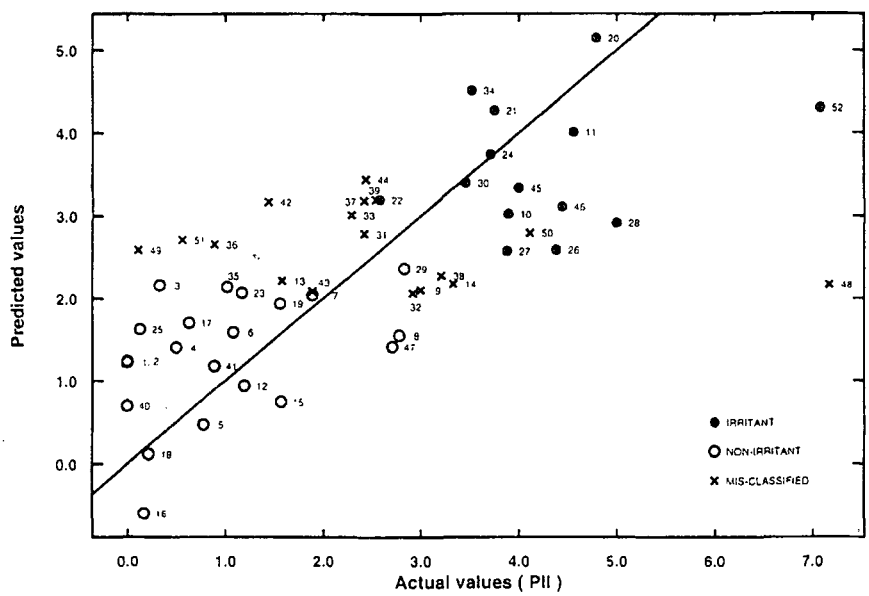

Fig. 1. Graph of the actual values of PII *v.* predicted for the 52 chemicals in Table 1. The actual irritant/non-irritant classifications of the chemicals, together with those chemicals misclassified by discriminant analysis, are also indicated on the plot. Points are numbered as in Table 1.

Table 3. Dataset of 40 electrophiles and two pro-electrophiles

Chemical	Class.	logP	MV (Å³)	Dipole moment (Debyes)
1. Ethyl triglycol methacrylate	NC	1.102	190.3	3.658
2. 2-Ethoxyethyl methacrylate	NC	1.268	122.7	3.656
3. 2,4-Decadienal	NC	3.532	128.7	4.763
4. 3,7-Dimethyl-2,6-nonadienal	NC	3.892	145.2	3.816
5. *cis*-Jasmone	NC	2.327	125.0	3.890
6. Ethyl thioethyl methacrylate	NC	1.948	133.4	4.021
7. Methacrolein	C	0.585	56.30	3.607
8. Ethyl tiglate	NC	2.245	103.5	2.130
9. 2,4-Hexadienal	C	1.517	75.83	4.744
10. Cinnamaldehyde	NC†	1.894	97.60	4.266
11. Isobutanal	NC†	0.363	64.90	2.779
12. Cyclamen aldehyde	NC	3.172	143.2	2.713
13. 2-Ethylhexanal	NC	2.507	118.6	2.805
14. Heptanal	NC	2.495	104.5	2.816
15. 3-Methylbutanal	NC†	1.348	78.40	2.827
16. Nonanal	NC	3.567	130.7	2.815
17. 2,5-Methylene-6-propyl-3-cyclohexenecarboxaldehyde	NC	3.403	145.1	2.708
18. 2-Phenylpropanal	NC	1.606	103.7	2.881
19. *p-iso*-Propyl-phenylacetaldehyde	NC	3.160	129.9	2.834
20. Hexylcinnamic aldehyde	NC	5.099	181.2	3.645
21. Hydroxycitronellal	NC	1.960	153.6	3.922
22. 4-Methylthiobenzaldehyde	NC	2.196	108.6	2.989
23. *p-tert*-Butyldihydrocinnamaldehyde	NC	3.996	157.7	2.802
24. 2,4-Dimethyl-3-cyclohexene-1-carboxaldehyde	NC	2.218	120.2	2.850
25. Salicylaldehyde	NC	1.876	88.85	3.425
26. 1-Bromo-4-chlorobutane	NC	2.572	98.40	0.058
27. 1,6-Dibromohexane	NC	3.776	128.4	0.003
28. 2-Bromopropane	NC	2.652	70.13	1.973
29. 1,3-Dibromopropane	NC	2.168	90.02	1.869
30. 2,3-Dichloropropionitrile	C	1.248	85.29	3.604
31. 2-Bromobutane	NC	3.188	81.24	1.951
32. 1-Bromohexane	NC	3.724	108.0	1.940
33. 1-Bromopentane	NC	3.188	94.78	1.953
34. Allyl bromide	C	1.726	65.15	1.801
35. Glycol bromoacetate	C	−0.909	101.3	3.881
36. 2-Hydroxyethyl acrylate	C*	−0.675	92.35	2.808
37. 2-Chlorobenzaldehyde	C*#	2.303	95.0	4.902
38. Acrolein	C*	−0.069	46.08	3.668
39. Formaldehyde	C*	−0.039	25.42	2.610
40. Methyl isothiocyanate	C*	0.252	55.13	0.986
41. Propargyl alcohol‡	C*	−0.474	47.05	1.408
42. 2-Butyn-1,4-diol‡	C*	−1.436	69.92	2.704

*Classifications taken from Annex I. C = corrosive; NC = non-corrosive.
†Chemicals misclassified by discriminant analysis; #misclassified by cross-validated discriminant analysis.
‡Pro-electrophiles.

Skin corrosivity

As a general rule, non-electrophilic neutral organic chemicals do not appear to be classified as corrosive. The author has found two exceptions to this rule, propargyl alcohol and 2-butyn-1,4-diol, which are listed as corrosive in Annex I (EEC, 1993). Both propargyl alcohol and 2-butyn-1,4-diol are highly toxic with LD_{50} (rat/oral) values of 70 mg/kg (Lenga, 1985) and 100–150 mg/kg (Richardson, 1992a), respectively. The source of this exceptional toxicity appears to be oxidation of the alcohol to the conjugated aldehyde, by analogy with allyl alcohol, which has been shown to be converted into acrolein by alcohol dehydrogenase in the liver (Richardson, 1992b). Allyl alcohol is similarly toxic—LD_{50} (rat/oral) of 71–105 mg/kg (Richardson, 1992b), cf. propanol LD_{50} (rat/oral) of 1870 mg/kg (Richardson, 1992c). Although these toxicity data arise from metabolic transformations carried out by the liver, there is every reason to believe that essentially all of these functions can also be performed in the skin (Hotchkiss, 1992; Martin *et al.*, 1987). For the purpose of this study, propargyl alcohol and 2-butyn-1,4-diol are considered to be pro-electrophiles.

The dataset of 40 electrophiles and two pro-electrophiles, their classifications (corrosive/non-corrosive) together with logP, molecular volume and dipole moment is shown in Table 3. Discriminant analysis of the dataset (Table 4) showed 39 out of the 42 chemicals to be well classified on the basis of only two parameters, (logP and molecular volume), the misclassified chemicals being one corrosive and two non-corrosives. Addition of the third parameter, dipole moment, did not increase the overall correlation, but resulted in all 12 of the corrosive chemicals being well classified with three non-corrosives—cinnamaldehyde, 3-methylbutanal and isobutanal—being misclassified. For predictive modelling of skin corrosivity, the latter situation is considered to be the more desirable. In this analysis, the variable 1/molecular weight (not shown in Table 3) was not significant. In contrast to the situation for skin irritation, corrosivity to skin was

Table 4. Discriminant analysis of 40 electrophiles and two pro-electrophiles

Step no.	Variable entering	Total fraction well classified	Well classified fraction per class: NC	C
1	logP (CHEMICALC)	0.833	0.867	0.750
2	MV	0.929	0.933	0.917
3	Dipole moment	0.929	0.900	1.0
Cross-validation confidence estimates:		0.929	0.933	0.917
Number in class:			30	12

C = corrosive NC = non-corrosive

Number of individuals predicted to belong to each class by final model (cross-validated prediction in parentheses)

	Predicted class: NC	C	Total
True Class:			
NC	27 (28)	3 (2)	30 (30)
C	0 (1)	12 (11)	12 (12)
Total	27 (29)	15 (13)	

Composition of discriminant axes

Variable	Axis Z1
logP (CHEMICALC)	0.903
Molecular volume	0.031
Dipole moment	−0.429

found to correlate with decreasing molecular volume, with increasing dipole moment, but with *decreasing* log P.

A three-dimensional plot of the three variables in Table 3 is shown in Fig. 2(A). From this plot it can be seen that there is a volume of parameter space defined by logP, molecular volume and dipole moment which is occupied by the corrosive chemicals

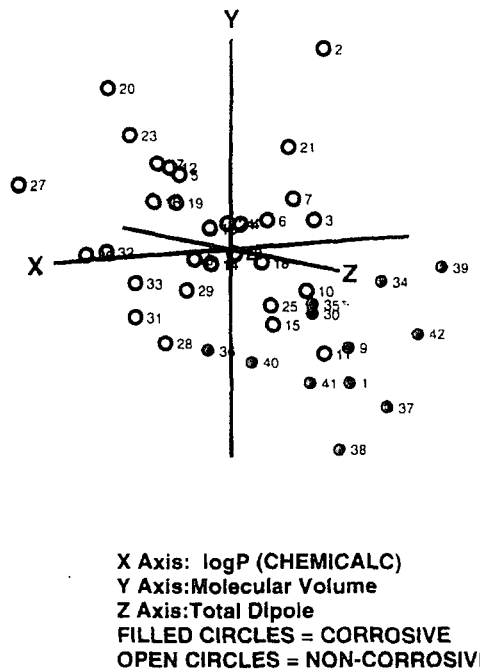

Fig. 2(A). Three-dimensional plot of logP, molecular volume and dipole moment for 40 electrophiles and two pro-electrophiles. Points are numbered as in Table 3.

in the dataset. This can be illustrated more clearly by submitting the same dataset to principal components analysis. In principal components analysis, the original variables are transformed into a new orthogonal set of linear combinants called principal components. The variance from the original descriptors is greatest in the first principal component, less in the second component and so on, allowing multicomponent datasets to be reduced to two- or three-dimensional plots without significant loss of information. The individual loadings of the three variables in the principal components analysis are summarized in Table 5. A plot of the first two principal components is shown in Fig. 2(B); in this case 89.9% of the total variance in the original three variables is expressed in a two-dimensional plot.

Chemicals classified as corrosive are clearly separated from those classified as non-corrosive; the three chemicals misclassified by discriminant analysis—cinnamaldehyde (10), isobutanal (11) and 3-methylbutanal (15)—are to be found on or near to the boundary between corrosives and non-corrosives.

It is clear from the analyses of the dataset in Table 3 that the chemicals classified as corrosive tend to be those with lower logP values and lower molecular volumes. This finding appears to conflict with the analysis for skin irritation which demonstrates an increase in irritancy with increasing logP corresponding to an increase in skin permeability. The origin of the apparent conflict lies in the fact that irritation and corrosivity are colligative properties of chemicals, that is, determined by the dose in terms of the number of molecules present, as well as being intrinsic properties. In order to make accurate comparisons between different chemicals, the toxico-

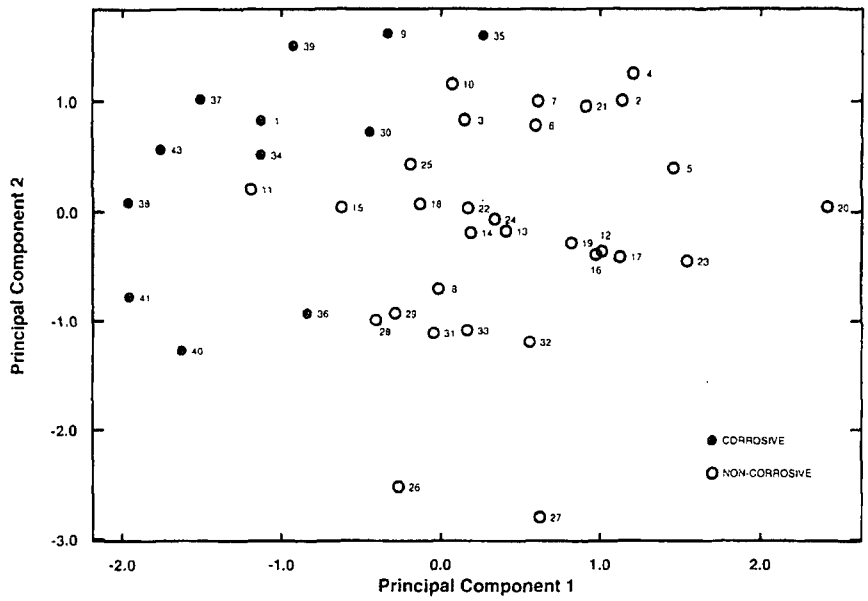

Fig. 2(B). Plot of the first two principal components of logP, molecular volume and dipole moment for 40 electrophiles and two pro-electrophiles. Points are numbered as in Table 3.

logical responses should ideally be expressed relative to molar doses, whereas in the Annex V method for skin irritation/corrosivity, a fixed mass or volume of chemical is applied. The dichotomy can be explained as follows.

Analysis of the skin irritation dataset (Table 1) is based on a hypothesis that irritancy is a function of skin permeability and dipole moment. The dataset contains only a few very irritant or corrosive chemicals, for which the QSAR works rather poorly. Inspection of Fig. 1 demonstrates that the PII values for small reactive chemicals such as heptanal (Table 1; no. 28), allyl bromide (48) and 2,4-hexadienal (52) are underpredicted by this model.

Analysis of the skin corrosivity dataset (Table 3) is based on the same variables that are used for the irritation dataset. From the outcome of the analysis, it is quite clear, however, that the key factor which discriminates the corrosive chemicals in the dataset from the non-corrosives, is the *molar* dose at which they were tested. Molar dose correlates inversely with molecular weight and within this dataset, molar dose is represented by two variables molecular volume and logP—which show correlations with molecular weights of 72.7% and 37.4%, respectively. This explanation is proposed for the fact that in this dataset the chemicals classified as corrosive tend to be those with lower logP values and lower molecular volumes. The presence of 10 additional corrosive chemicals in the corrosivity dataset compared with the irritation dataset biases the distribution of molecular volumes and logP values in the former towards lower values. The hyperbolic nature of the relationship between dose and molecular volume results in the greater sensitivity of the corrosivity dataset to the 'dose effect'.

The validity of using dipole moment as a reactivity parameter for neutral organic chemicals has been demonstrated previously in a QSAR for eye irritation (Barratt, 1995c); it therefore seems reasonable that the same parameter should also be valid for modelling the skin irritation potential of neutral organic chemicals. Using the same argument as for neutral organic chemicals, dipole moment can be expected to model that part of the reactivity of electrophiles which arises from physicochemical disruption of ion permeability. Clearly, it will not account for the electrophilic reactivity of electrophiles—the more reactive the electrophile, the poorer the correlation.

The energy of the lowest unoccupied molecular orbital (E_{LUMO}), calculated using the semi-empirical molecular orbital programme MOPAC with the PM3 Hamiltonian (Stewart, 1989) in Sybyl, was also

Table 5. Principal components vectors

	Principal Comp. 1	Principal Comp. 2	Principal Comp. 3
logP (CHEMICALC)	0.670	−0.348	0.656
Molecular volume	0.716	0.068	−0.692
Dipole moment	0.197	0.935	0.295
Fraction of variance explained:	0.552	0.347	0.101
Total fraction of variance explained:	0.552	0.899	1.00

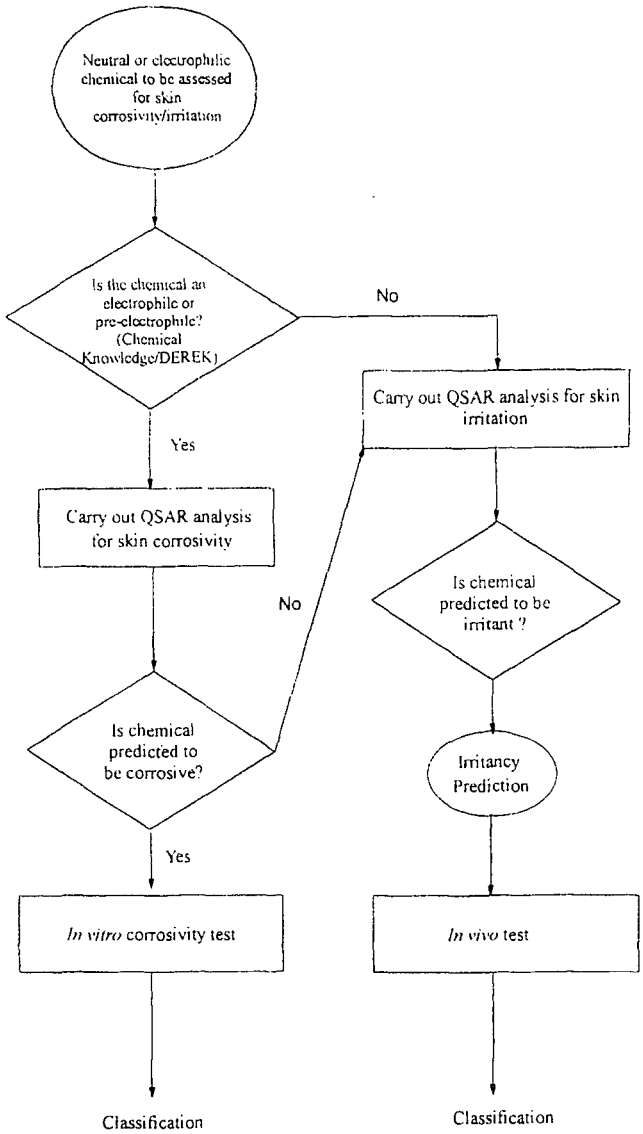

Fig. 3. Scheme for the prediction of skin corrosivity or irritation potentials of neutral and electrophilic organic chemicals.

examined as a reactivity parameter for both datasets. E_{LUMO} is a parameter widely used in QSAR studies as a measure of electrophilic reactivity, for example, Eriksson *et al.* (1994). E_{LUMO} was found not to be a statistically significant variable ($F < 4.0$) in the stepwise regression analysis for skin irritation and in the discriminant analysis for skin corrosivity. In the discriminant analysis for skin irritation, inclusion of E_{LUMO} resulted in a small increase in the number of chemicals correctly classified from 28 to 31; however, increased irritancy to skin was found to correlate with increasing values of E_{LUMO}. Since *decreasing* values of E_{LUMO} are known to correlate with *increased* electrophilic reactivity, the small improvement in correlation found in the present case was presumed to be by chance and was discarded.

The use of dipole moment in these analyses represents an approximation, albeit one which works reasonably well. A more practical solution to obtaining reactivity parameters is to measure the cytotoxicity of the chemicals using *in vitro* toxicology techniques. An example of this approach is the use of neutral red uptake data to measure the cytotoxicity of electrophilic organic acids. These data, in combination with parameters used in a QSAR study of the corrosivity of organic acids (Barratt, 1995a), are proving valuable in discriminating between chemicals with the EC classifications R34 (Corrosive: causes burns) and R35 (Corrosive: causes severe burns) (Barratt *et al.*, 1996). The same approach is to be used for the chemicals described in this paper.

A stepwise approach to the classification of corrosives and irritants

A scheme illustrating a stepwise approach for the classification of new or untested neutral and electrophilic organic chemicals is shown in Fig. 3. The first stage in the process is to identify whether or not the chemical falls into the correct classification to be assessed by the scheme. Chemicals which are excluded are inorganics and organometallics, organic salts, surfactants, and organic acids, bases and phenols; QSARs suitable for the classification of this latter group are published elsewhere (Barratt, 1995a). The next step is to identify whether the chemical is an electrophile (or pro-electrophile) or a neutral organic chemical; this can be achieved either using knowledge of organic chemistry or by the use of an expert system, such as DEREK (Sanderson and Earnshaw, 1991). The DEREK expert system contains a rulebase of chemical structural alerts for the reactivity component of skin sensitization (Barratt and Basketter, 1994; Barratt *et al.*, 1994), which consist predominantly of electrophilic functions.

If the chemical is identified as an electrophile or pro-electrophile, its potential for skin corrosivity is examined using the QSARs for corrosivity. If QSAR evidence suggests that the chemical should be classified as 'corrosive', the classification can be confirmed by carrying out an *in vitro* corrosivity test such as the transepidermal electrical resistance test (Oliver *et al.*, 1986 and 1988) and labelled accordingly. If the chemical is considered to be non-corrosive on the basis of QSAR evidence, its potential for skin irritation is examined using the QSARs for skin irritation.

If the chemical is not an electrophile or pro-electrophile, it can be assumed to be non-corrosive (as a general rule, non-electrophilic neutral organic chemicals do not appear to be classified as corrosive), and its potential for skin irritation is examined using the QSARs for skin irritation. Current legislation does not permit the use of non-animal procedures for the classification and labelling of chemicals for skin irritation, therefore the next step in the process is to conduct an animal test.

The QSARs presented above are expected to give reasonable predictions of the skin corrosivity potential of neutral and electrophilic organic chemicals with the potential to be used initially as a pre-screen prior to carrying out animal procedures for the classification of new chemicals. Prediction of the skin irritation potentials for some non-irritant and irritant neutral and electrophilic organic chemicals using these techniques is also possible. Because there exists a broad band of biological uncertainty around the irritant/non-irritant classification boundary, no firm prediction of classification is possible for the large number of chemicals whose skin irritation potentials fall in this region. This band of biological uncertainty is largely ignored in practice by the simple expedient of accepting the biological data at their face value.

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5

Dissociation Constants:
pKa = 4.895 at 25 deg C
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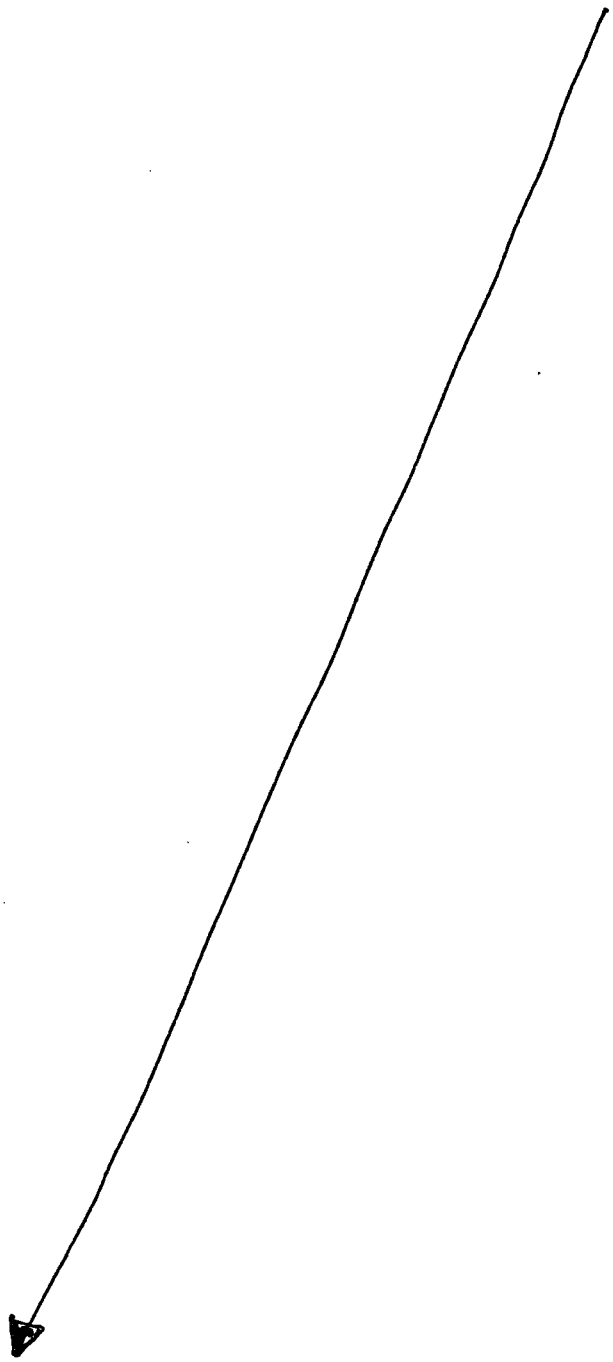

REFERENCE 6

United States Environmental Protection Agency | Office of Prevention, Pesticides And Toxic Substances (7508W) | EPA-738-F-92-013 September 1992

6

EPA **R.E.D. FACTS**

Soap Salts

Pesticide Reregistration

All pesticides sold or used in the United States must be registered by EPA, based on scientific studies showing that they can be used without posing unreasonable risks to people or the environment. Because of advances in scientific knowledge, the law requires that pesticides which were first registered years ago be reregistered to ensure that they meet today's more stringent standards.

In evaluating pesticides for reregistration, EPA obtains and reviews a complete set of studies from pesticide producers, describing the human health and environmental effects of each pesticide. The Agency imposes any regulatory controls that are needed to effectively manage each pesticide's risks. EPA then reregisters pesticides that can be used without posing undue hazards to human health or the environment.

When a pesticide is eligible for reregistration, EPA announces this and explains why in a Reregistration Eligibility Document, or RED. This fact sheet summarizes the information in the RED for soap salts.

Use Profile

Soap salts include the two pesticide active ingredients potassium salts of fatty acids (including potassium laurate, potassium myristate, potassium oleate and potassium ricinoleate), and ammonium salts of fatty acids (ammonium oleate).

Potassium salts of fatty acids are used as insecticides, acaricides, herbicides and algaecides. They are used to control a variety of insects and mosses, algae, lichens, liverworts and other weeds, in or on many food and feed crops, ornamental flower beds, house plants, trees, shrubs, walks and driveways, and on dogs, puppies and cats.

Ammonium salts of fatty acids are used as a rabbit and deer repellent on forage and grain crops, on vegetables and field crops, in orchards, and on nursery stock, ornamentals, flowers, lawns, turf, vines, shrubs and trees.

Regulatory History

The first pesticide product containing soap salts as an active ingredient was registered in 1947. Currently, 24 registered pesticide products contain soap salts. Some of these products also contain other active ingredients.

When EPA published reregistration List D in the Federal Register on May 5, 1990, Soap Salts (case 4083) contained eight active ingredients. However, four of these were combined into the single active ingredient, potassium salts of fatty acids; three others were cancelled and are no longer subject to reregistration. Thus, two active ingredients remain.

Potassium salts of fatty acids used on food and feed crops have been exempted from the requirement of a tolerance (or maximum residue limit) for all raw agricultural commodities since 1982 (please see 40 CFR 180.1068). They are generally recognized as safe (GRAS) by the Food and Drug Administration (FDA) (please see 21 CFR 172.863). Although ammonium salts of fatty acids also were exempted from tolerance requirements in 1982, public notice was not provided. To correct this oversight, EPA will publish an appropriate Federal Register notice soon.

In 1988, EPA determined that soap salts have "no independent pesticidal activity" in antimicrobial products, and must be classified as inert ingredients in those products (please see 40 CFR 153.139.) Therefore, antimicrobial pesticides that contain soap salts as inert ingredients are not subject to this RED. Antimicrobials that still contain soap salts as active ingredients are considered misbranded and are subject to misbranding enforcement action or cancellation.

Human Health Assessment

Toxicity

Soap salts are of low toxicity when taken orally or exposed briefly to the skin, and have been placed in Toxicity Category IV (indicating the lowest level of toxicity) for these acute effects. However, they can cause mild or moderate irritation when exposed to the skin for longer periods of time. Ammonium salt products also can cause permanent eye damage.

Fatty acids normally are metabolized, forming simple compounds that serve as energy sources and structural components used in all living cells. However, soap salts caused reproductive and mutagenic effects when administered to laboratory animals at high doses.

Dietary Exposure

Although people could be exposed to low levels of soap salts on foods, these residues pose no known health risks. Soaps are mineral salts of naturally occurring fatty acids. These fatty acids are a significant part of the normal daily diet. Residues from the pesticide uses of soap salts are not likely to exceed levels of naturally occurring fatty acids in commonly eaten foods. Again, both potassium and ammonium salts of fatty acids are exempted from tolerance requirements, and potassium salts are generally recognized as safe by FDA.

Occupational and Residential Exposure

People applying soap salts may be exposed to these compounds. Potassium salts are of low toxicity to humans, and there is no reason to

expect that reasonable use will constitute any significant hazard. However, protective eyewear is required while applying the ammonium salts of fatty acids to prevent permanent eye injury.

Human Risk Assessment

Soap salts are of low acute toxicity when taken orally or exposed to the skin. Residues from the pesticide uses of soap salts are not likely to exceed levels of naturally occurring fatty acids in commonly eaten foods. Thus, EPA believes the risks of the soap salts to applicators and consumers are negligible. The risk of eye injury to applicators of the ammonium salts can be mitigated by use of protective eyewear.

Environmental Assessment

Environmental Fate

Hydrolysis of potassium salts of fatty acids (and probably also of ammonium salts) does not occur over a period of 43 days. The half-life of these fatty acids is estimated to be less than one day. As can be expected, microbial organisms rapidly degrade fatty acids in soil. Soap salts cannot dissipate totally in soil, however, because soil has a natural content of fatty acids resulting from plant metabolism and microbial action. Fatty acids are a significant part of the normal daily diet of mammals, birds and invertebrates.

Ecological Effects

Ammonium salts of fatty acids are used outdoors as a rabbit and deer repellent. They are practically non-toxic to upland game birds and waterfowl. Their other potential hazards were estimated using data from the potassium salts.

Acute and subacute toxicity studies using potassium salts of fatty acids indicate that soap salts are relatively non-toxic to birds. They are slightly toxic to both coldwater and warmwater fish species. The potassium salts are highly toxic to aquatic invertebrates.

No studies regarding the effects of the soap salts on non-target insects were available for review. Such studies still are required. In addition, product use rate information is needed to confirm that soap salts pose a minimal threat to endangered species.

Environmental and Ecological Risk Assessment

Pesticides containing potassium or ammonium salts of fatty acids are used on a wide array of outdoor sites. Once applied, however, the soap salts are degraded quickly in soil by microbes, and do not persist in the environment. The soap salts pose minimal risks to birds and are only slightly toxic to fish. They are highly toxic to aquatic invertebrates. However, since soap salts are not applied directly to water, their current uses should not seriously impact aquatic invertebrates. Additional studies are needed to assess their effects on non-target insects. The soap salts should pose minimal threats to endangered species. In summary, based on

the data reviewed, EPA finds that the soap salts will not cause unreasonable adverse effects on the environment.

Additional Data Required

EPA has waived all generic data requirements for the soap salts except basic product identity and chemistry studies and acute ecotoxicity studies, which were submitted and reviewed. EPA is requiring honeybee toxicity data for potassium salts and confirmatory ecotoxicity studies on fish and aquatic invertebrates for ammonium salts of fatty acids to confirm that these pesticides do not pose significant ecological hazards. Product-specific chemistry and acute toxicology studies also are required for reregistration.

Product Labeling Changes Required

The labels of all registered soap salts products must comply with EPA's current pesticide labeling requirements. In addition,

- Products with outdoor uses and the manufacturing use product must bear the following label statement: "This product may be hazardous to aquatic invertebrates. Do not apply directly to water, areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water by cleaning of equipment or disposal of water."
- Labeling of all products must include appropriate precautionary statements.
- Labeling of ammonium salts products must require use of protective eyewear (safety glasses, goggles or faceshield).
- Labels must upgrade the ingredients statement by declaring potassium salts or ammonium salts of fatty acids, rather than "soap".
- Labels of products for crop uses must be upgraded by stating specific crops and/or crop groups.

Regulatory Conclusion

- All registered pesticide products containing the active ingredient soap salts are not likely to cause unreasonable adverse effects in people or the environment, and are eligible for reregistration. These products will be reregistered once the required additional generic data, product-specific data and revised labeling are received and accepted by EPA.
- Registered products containing soap salts as well as other active ingredients will be reregistered once the other active ingredients also are determined to be eligible for reregistration.

For More Information

EPA is requesting public comments on the Reregistration Eligibility Document (RED) for soap salts during a 60-day time period, as announced in a Notice of Availability published in the Federal Register. To obtain a copy of the RED or to submit written comments, please contact the Public

Response and Program Resources Branch, Field Operations Division (7506C), Office of Pesticide Programs (OPP), US EPA, Washington, DC 20460, telephone 703-305-5805.

In the future, the soap salts RED will be available from the National Technical Information Service (NTIS), 5285 Port Royal Road, Springfield, VA 22161, telephone 703-487-4650.

For more information about soap salts or about EPA's pesticide reregistration program, please contact the Special Review and Reregistration Division (7508W), OPP, US EPA, Washington, DC 20460, telephone 703-308-8000. For information about reregistration of individual soap salts products, please contact PM Team 14, Registration Division (7505C), OPP, US EPA, Washington, DC 20460, telephone 703-305-6600.

For information about the health effects of pesticides, or for assistance in recognizing and managing pesticide poisoning symptoms, please contact the National Pesticides Telecommunications Network (NPTN). Call toll-free 1-800-858-7378, 24 hours a day, seven days a week, or fax your inquiry to 806-743-3094.

REFERENCE 7

Reported uses (ppm): (FEMA, 1994) *(Continued)*

Food Category	Usual	Max.
Baked goods	17.56	24.52
Frozen dairy	9.88	13.50
Nonalcoholic beverages	5.63	8.52
Soft candy	8.40	15.94

(Part 2 of 2)

Synthesis: From decanal and methyl alcohol.
Aroma threshold values: n/a
Taste threshold values: n/a
Natural occurrence: Not reported found in nature.

DECANOIC ACID

Synonyms: Decylic acid; **Butter acids**; **Capric acid**; *n*-Capric acid; Caprinic acid; Caprynic acid; **Decanoic acid (8CI)(9CI)**; *n*-Decanoic acid; Decoic acid; *n*-Decoic acid; *n*-Decylic acid; neo-Fat 10; 1-Nonanecarboxylic acid

CAS No.:	334-48-5	FL No.:	08.011	FEMA No.:	2364	NAS No.:	2364
CoE No.:	11	EINECS No.:	206-376-4	JECFA No.:	105		

Description: Fatty, unpleasant, rancid odor.
Consumption: Annual: 18833.33 lb Individual: 0.01596 mg/kg/day
Regulatory Status:
CoE: Approved. Bev.: 10 ppm; Food: 10 ppm
FDA: 21 CFR 178.1010
FDA (other): n/a
JECFA: ADI: Acceptable (no safety concern at current levels of intake) (1997)
Trade association guidelines: FEMA PADI: 1.814 mg IOFI: Nature Identical
Empirical Formula/MW:
$C_{10}H_{20}O_2$/172.27 $CH_3—(CH_2)_8—COOH$

Specifications: (JECFA, 1997)

Acid value	320-329 (max)
Appearance	White crystals
Boiling point	268°C
Solubility	Soluble in most organic solvents; practically insoluble in water
Specific gravity	0.893

Reported uses (ppm): (FEMA, 1994)

Food Category	Usual	Max.
Baked goods	9.56	12.39
Cheese	10.70	10.80
Chewing gum	0.01	0.01
Fats, oils	4.47	8.97
Frozen dairy	1.61	7.45
Gelatins, puddings	0.49	2.06
Gravies	0.30	0.60
Imitation dairy	7.00	14.00
Meat products	1.89	2.00
Nonalcoholic beverages	0.98	1.57
Snack foods	2.00	4.00
Soft candy	1.90	6.13

Synthesis: Prepared by oxidation of decanol.
Aroma threshold values: Detection: 2.2 to 102 ppm
Taste threshold values: n/a
Natural occurrence: Reported found in apple, beer, preferments of bread, butter, oil, cheese, blue cheese, Romano cheese, cheddar cheese, Roquefort cheese, roasted cocoa bean, cognac, muscat grape, grape musts and wine, and other natural sources. Also reported in citrus peel

oils, orange juice, apricots, guava, papaya, strawberry, butter, yogurt, milk, mutton, hop oil, bourbon and Scotch whiskey, rum, coffee, mango and tea.

1-DECANOL

Synonyms: Alcohol C-10; Capric alcohol; Decyl alcohol; Nonyl carbinol; Alcohol C10; C 10 Alcohol; Caprinic alcohol; Decanol; ***n*-Decanol**; **1-Decanol (9CI)**; **Decan-1-ol**; *n*-Decan-1-ol; *n*-Decatyl alcohol; **Decyl alcohol (8CI)**; Decyl, *n*-alcohol; ***n*-Decyl alcohol**; Decylic alcohol; Nonylcarbinol; *n*-Nonylcarbinol; Primary decyl alcohol

CAS No.:	112-30-1	FL No.:	02.024	FEMA No.:	2365	NAS No.:	2365
CoE No.:	73	EINECS No.:	203-956-9	JECFA No.:	103		

Description: 1-Decanol has a floral odor resembling orange flowers and a slight, characteristic fatty taste.

Consumption: Annual: 135.00 lb Individual: 0.0001144 mg/kg/day

Regulatory Status:

CoE: Approved. Bev.: 2 ppm; Food: 10 ppm

FDA: 21 CFR 172.515

FDA (other): n/a

JECFA: ADI: Acceptable (no safety concern at current levels of intake) (1997)

Trade association guidelines: FEMA PADI: 7.742 mg IOFI: Nature Identical

Empirical Formula/MW:

$C_{10}H_{22}O$/158.28 $CH_3—(CH_2)_8—CH_2OH$

Specifications: (JECFA, 1997)

Acid value	1.0 (max)	Refractive index	1.435-1.439
Appearance	Colorless liquid	Solubility	1:3 in 60% alcohol; soluble in alcohol, ether, mineral oil, propylene glycol and most fixed oils; insoluble in glycerin
Assay	98% (min)	Specific gravity	0.826-0.831
Boiling point	233°C		

Reported uses (ppm): (FEMA, 1994)

Food Category	Usual	Max.	Food Category	Usual	Max.
Alcoholic beverages	2.00	5.00	Gelatins, puddings	24.78	27.97
Baked goods	37.11	40.21	Hard candy	0.60	0.79
Chewing gum	0.25	0.25	Nonalcoholic beverages	12.39	17.01
Frozen dairy	26.05	32.75	Soft candy	21.43	25.27

Synthesis: Synthetically prepared from coconut oil derivatives; by reduction of some capric esters, such as methyl caprate.

Aroma threshold values: Detection: 6 to 47 ppb

Taste threshold values: n/a

Natural occurrence: Reported in the essential oils of ambrette seeds and almond flowers; also in citrus oils, fermented beverages, apple juice, bilberry, American cranberry, papaya, raspberry, cheeses, milk, butter, beef, pork, beer, cognac, whiskey, red, white and sparkling wines, coriander seeds and cardamom.

REFERENCE 8

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Chemistry of New Industrial Oilseed Crops

Robert Kleiman

INTRODUCTION

The attraction for developing new oilseeds is several-fold. First, a new crop can provide a significant increase in the level of income for the farmer; second, new crops can contribute to the alleviation of negative balance of payments; and third, they can provide materials that are critical to our national interests, especially if we at present depend on imported materials. The reason we select specific plant species for development is the difference in chemical functionality of the proposed new seed oil from the oils we now have domestically available. This paper deals with only a few of the plants available from the wild that have potential as new sources of industrial materials. The pertinent chemical background of some species that are now cultivated experimentally in the United States will be presented.

NEW OILSEED CROPS

Sources of Medium Chain Fatty Acids—*Cuphea*

The basic source of medium chain fatty acids is tropically-grown coconut and palm kernel oil. The U.S. has no domestic source of these materials and therefore imports about 500,000 tonnes (one billion pounds) annually of "lauric" oils.

However, many species from the genus *Cuphea* (Lythraceae) have potential as sources of medium chain triglycerides (Wilson et al. 1960, Miller et al. 1964, Wolf et al. 1983, Graham and Kleiman 1985, Graham et al. 1981). These plants are native to the New World, from Southern U.S. to Northern South America. Most are herbaceous annuals that will grow in many locations. However, *Cuphea* is only a few years from the wild and still has the characteristics of a wild plant. Those characteristics that differ from cultivated plants are its propensity to seed shatter, its indeterminate flowering nature, and its overall stickiness. If these wild traits can be overcome, *Cuphea's* chemistry, coupled with the annual and therefore renewable nature of the plant, certainly can make it a new crop.

Table 1 illustrates the diversity in fatty acid composition available in *Cuphea* germplasm. While there is some variation from accession to accession, the table shows species that are rich in specific single fatty acids. *Cuphea painteri,* for instance, is very rich in caprylic (8:0) acid (73%) while *C. carthagenensis* has lauric acid (12:0), as its major fatty acid (81%). *Cuphea koehneana* is probably the best example of a monoacid seed oil, with more than 95% of its acyl groups as capric acid. As a source for lauric acid, *Cuphea* ssp. have more to offer than coconut oil (Table 1), because the concentration of lauric acid in the oil is potentially much greater. Isolation of single fatty acids should be easily accomplished and tailor-made fatty acid compositions should be possible.

Oil and protein values were determined only on a handful of accessions because only a few seeds have been collected from many of the species. In those species, oil percent varied from 16 to 42%. Protein (%N x 6.25) levels were from 15 to 24% of the whole seed. Table 2 shows the amino acid composition of two *Cuphea* species.

The triglyceride analysis of *C. lanceolata* (Litchfield et al. 1967) shows that the combination of acyl groups within triglyceride molecules is not the result of random distribution but the specific combination of certain fatty acids, particularly C_{10}. Gas chromatography of the intact triglycerides show that the C_8 was completely contained in the group with carbon number of C_{28} indicating that the C_{30} peak was made up of three C_{10} fatty acids and not a combination of C_8, C_{10}, and C_{12}. These combinations have significance when *Cuphea* oils are to be used in nutritional and medical applications.

Other members of the Lythraceae have been examined to see if fatty acid compositions are similar to *Cuphea* (Graham and Kleiman 1987). However, only small amounts of medium chain fatty acids were found.

Seed Oils of the Apiaceae (Umbelliferae)

While the seeds oils from the plants of the Apiaceae (Umbelliferae) do not contain medium chain fatty acids per se, most do contain large amounts of petroselinic (*cis*-6-octadecenoic) acid. This positional isomer of the common oleic (*cis*-9-octadecenoic) acid can be cleaved at the double bond to form two industrially useful materials, adipic and lauric acids (Fig. 1). The method of choice to perform this type of cleavage is ozonolysis. For example, the production of pelargonic and azeleic acids is from the ozonolysis of oleic acid. However, the production of lauric acid by this means will have to compete with lauric acid from a much cheaper starting material, coconut oil.

With the possible exception of dill, most Umbelliferae are not produced in the U.S. for their seed oil. Production of such crops as celery, dill, carrots is for food and or condiments. However, seed yields for common fennel of over 2800 kg of seed per hectare have been reported. With agronomic and breeding research, higher seed yields and oil content should be achieved. Wild species of the Apiaceae have been reported that have as much as 40% seed oil containing 80% petroselenic acid (Kleiman and Spencer 1982).

Meadowfoam (*Limnanthes alba*)

The oil composition from meadowfoam seed (Table 3, Fig. 2) is unique in several ways. First, over 95% of its acyl groups are longer than C18; secondly, about 90% of these fatty acids have double bonds in the delta-5 position; and thirdly, there are essentially no polyunsaturates (Smith et al. 1960, Bagby et al. 1961, Phillips et al. 1971). Along with the 60% *cis*-5-eicosenoic acid, the 8% *cis*-5-docosenoic acid, and the 10% erucic acid one finds 15-30% of $22:2^{5,13}$. The latter is a polyunsaturated dienoic acid, but six carbon atoms separate the double bonds, thus making the acid react essentially as a monoenoic fatty acid in terms of its oxidative stability.

The oil should be oxidatively stable, not only because of the lack of polyenoic fatty acids and its long chain nature but also because the delta-5 bond is more stable than olefins with the double bond in the center of the fatty acid molecule (Kaneniwa et al. 1988). The *cis*-5 bond also allows lactonization to proceed easily, especially after epoxidation (Fore and Sumrell 1966) (Fig. 3 and 4). Oxidative cleavage of the $20:1^5$ at the double bond results is glutaric and pentadecanoic acids. The use of the latter 15:0 acid should be evaluated because it is not now industrially available. The $22:2^{5,13}$ has been successfully epoxidized (Carlson et al. 1989). The reactive diepoxide should be a useful industrial intermediate.

Natural meadowfoam oil is in the form of triglycerides. However, they have been converted to liquid wax-esters by reduction of fatty acids to alcohols and reesterification with the unreacted fatty acids (Miwa 1972, Nieschlag et al. 1977). These C_{40} and C_{42} molecules should be useful, both sulfurized and unreacted, in lubricants.

Meadowfoam (*Limnanthes alba*) is a member of the Limnanthaceae. Though the presence of the sulfur containing glucosinolates is usually associated with the Brassicaceae (Cruciferae), meadowfoam seed contains significant levels of glucosinolates that upon hydrolysis yield both a volatile mustard oil, *m*-methoxybenzyl isothiocyanate, and the nonvolatile 5,5-dimethyloxazolidine-2-thione (Daxenbichler and VanEtten 1974) (Fig. 5). These products must be considered when meadowfoam defatted meal is used as animal feed. Meadowfoam is grown as a winter annual principally in the Willamette Valley of Oregon where yields of over 1.1 t/ha are routine (Jolliff, pers. commun.). Breeding and agronomic research are ongoing at Oregon State University and at other locations. The genus is native to southern Oregon and northern California.

Lesquerella

Plants of the genus *Lesquerella* are members of the Brassicaceae (Cruciferae) and are native to North America. We became interested in these plants when we found that their seed oils are rich in hydroxy fatty acids (Smith et al. 1961, Mikolajczak et al. 1962, Smith et al. 1962, Kleiman et al. 1972).

Castor oil is the only commercial source of hydroxy acids, and the fatty acid in the oil is ricinoleic, 12-hydroxy *cis*-9-octadecenoic, acid, found at the 85% level. Though most *Lesquerella* species have small amounts of this acid, large concentrations of other hydroxy acids are found. The structures of these compounds are shown in Fig. 6. Though most species in this genus have several hydroxy fatty acids, they are generally rich in only one. Table 4 illustrates the different types of *Lesquerella* compositions. The dienoic hydroxy fatty acids, densipolic and auricolic, have potential as fatty intermediates and tung oil replacement. However, we are presently concerned with the high lesquerolic acid types. At the USDA's Water Conservation Laboratory, Phoenix, Arizona, agronomic and breeding research is now underway on one of these, *Lesquerella fendleri*. Yields of better than 1500 kg/ha have been achieved in just a few years (A.E. Thompson, pers. commun.). With continued work in this area increases in seed yields, lesquerolic acid, and oil content are expected.

The structure of lesquerolic acid is homologous to ricinoleic acid. It has two additional carbon atoms at the carboxyl end of the molecule. This similarity allows many of the same reactions and, presumably, the same uses as for the castor-based fatty acid. This includes the production of 2-octanol and 2-octanone, depending on reaction temperature during alkali cleavage of the acid. However, this same reaction produces dodecanedioic and hydroxydodecanoic acids from lesquerolic acid instead of the analogous acids from ricinoleic that are two carbons shorter. The C_{12} dibasic acid, dodecanedioic acid, is the basis for nylon 1212 (Fig. 7). The current raw material for production of dodecanedioic acid is petroleum. Without alkali, a major product from thermal fragmentation of ricinoleic acid is 10-undecenoic acid (Naughton et al. 1979) (Fig. 8). This material, after bromination and subsequent amination, is the starting material for nylon-11. The analogous 12-tridecenoic acid is produced from

lesquerolic acid (Fig. 8) and could be used in the production of the monomer for nylon-13. The saturated, via hydrogenation, hydroxy acids, as their lithium soaps, could be useful in the production of greases. The intact triglycerides from *Lesquerella* should be suitable for many uses. For example, they could substitute for dehydrated and oxidized castor oil. Many of the properties may be enhanced over those of castor oil because of the increased chainlength of this new crop oil.

Like most of its cruciferous relatives, *Lesquerella's* seed meal contains glucosinolates. There are several different glucosinolates identified in *Lesquerella* spp. (Daxenbichler et al. 1962). Many of these can be removed easily from the meal if converted to the volatile isothiocyanate (Daxenbichler et al. 1961). The amino acid composition of the seed meal has been reported (Miller et al. 1962a). Its especially high lysine content makes it attractive as a protein supplement.

Jojoba (*Simmondsia chinensis*)

Made up almost exclusively of liquid wax-esters, the oil of jojoba seed is unique in the plant kingdom (Wisniak 1987). The general structure of these lipids is shown in Fig. 9. Jojoba is often touted as a whale oil substitute, while in fact it has features that, in most applications, make it superior. First of all, whale oil has a significant (about 30%) amount of polyunsaturated triglycerides as part of its total lipids. These make it oxidatively less stable than jojoba, which has essentially no triglycerides, polyunsaturated or otherwise. Secondly, jojoba wax-esters are considerably longer in chainlength, ranging from C_{40} to C_{44}. The bulk of sperm whale oil has chainlengths of C_{32} to C_{36}. The fatty acid and alcohol compositions, making up the wax-esters of jojoba oil, are listed in Table 5. The specific combinations of alcohols and acids have been established by mass spectrometry (Spencer et al. 1977).

Jojoba oil's current use is centered on the cosmetic and personal care industry This is due primarily to its ability to lubricate without the sense of greasiness. There are about 6,000 productive hectares (15,000 acres) of jojoba in the southwest U.S. As these areas become more fruitful, and additional areas come on-line, the price of the oil is expected to decrease. As this comes about, more industrially oriented uses for this material will emerge in the market place. A number of derivatives have been made. These include sulfurized and halogenated jojoba oil, for high-pressure applications such as in automobile transmissions, and hydrogenated jojoba as a wax.

The whole jojoba seed contains about 15% protein (N x 6.25). When considering the large amount of oil in the seed (50%), the defatted meal has about 30% protein. However, the seed contains also about 11% antinutritional compounds. These are simmondsin, simmondsin 2'-ferulate, 5-desmethylsimmondsin, and didesmethylsimmondsin (Wisniak 1987) as illustrated in Fig. 10. Several methods are now under development to eliminate these materials in order to use the meal as a nutritional animal feed.

High-Erucic Acid Sources

Crambe (White and Higgins 1966) *(Crambe abyssinica)* and industrial rape (Ackman 1983) (either *Brassica napus* or *B. campestris)* are potential sources for erucic acid in the U.S. While European rape has long been imported into this country, it is recently being introduced as a new crop for the U.S. Crambe has been suggested for many years as an erucic acid source and has been grown intermittently on small areas. All three species are members of the Brassicaceae (Cruciferae).

Erucic (*cis*-13-docosenoic) acid is now converted to erucamide for use as a slip agent in the manufacture of polyethylene sheets. The acid also has been cleaved to form brassylic and pelargonic acids. The brassylic acid has been used to produce nylon 1313. Both erucic acid itself and its cleavage product, brassylic acid, have potential in other commercial products such as plasticizers, lubricants, and surfactants.

The seed meals of these crops have high protein levels and good amino acid compositions (Miller et al. 1962) (See Table 3). While the glucosinolates in rapeseed can be eliminated through breeding, no germplasm is yet available to lower the 8% glucosinolates in crambe seed. However, crambe meal has been cleared for use at the 5% level in animal feeds.

Epoxy Oils

Many plants produce seed oils with epoxy fatty acids (Earle 1970). A few produce as much as 80% of one epoxy acid, vernolic (12,13-epoxy-*cis*-9-octadecenoic) acid. Those with potential as crops are *Vernonia galamensis* (Perdue et al. 1986), Stokes Aster (Earle 1970), and *Euphorbia lagascae* (Kleiman et al. 1965). The fatty acid compositions of the seed oils of these species are given in Table 6. None of these species are now grown in the U.S. However, *Vernonia galamensis* is grown experimentally in its native equatorial Africa. Until recently, the long daylight hours of the U.S. did not allow flowering of this species. Workers at the Water Conservation Laboratory have now grown a few lines whose flowering pattern looks promising for that area. Stokes aster (*Stokesia laevis*), a southeast U.S. herbaceous perennial, has been grown experimentally with promising results (T.A. Campbell, pers. commun.). *Euphorbia lagascae* is naive to southern Europe and is being considered as a crop there.

These epoxy oils have an advantage over commercially epoxidized oils in that the location, number, and configuration of epoxy and olefinic groups are rigorously known. The oil of *Vernonia galamensis* forms excellent baked coatings on steel (Carlson et al. 1981) and interpenetrating polymer networks with other polymers (Sperling et al. 1983). These oils have potential in the production of plastics, paints, and lubricants.

Other Potential Species

I have tried to summarize the chemical aspects of the new industrial oil seed crops currently under exploration in the United States. There are a number of other proposed crops that are not now being looked at here but should be mentioned. *Dimorphotheca pluvialis* produces an oil rich in dimorphecolic acid (Earle et al. 1962), a C_{18} conjugated dienol, and *Crepis alpina* (Spencer et al. 1969) is rich in crepenynic acid, an acetylenic acid. Hopefully, in the future we can take greater advantage of the diversity of nature and use American produced renewable raw materials for our industrial needs.

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Table 1. Fatty acid composition of some *Cuphea* seed oils.

	Distribution (% of total fatty acids)				
Species	8:0 caprylic	10:0 capric	12:0 lauric	14:0 myristic	Others
C. painteri	73.0	20.4	0.2	0.3	6.1
C. hookeriana	65.1	23.7	0.1	0.2	10.9
C. koehneana	0.2	95.3	1.0	0.3	3.2
C. lanceolata		87.5	2.1	1.4	9.0
C. viscosissima	9.1	75.5	3.0	1.3	11.1
C. carthagenensis		5.3	81.4	4.7	8.6
C. laminuligera		17.1	62.6	9.5	10.8
C. wrightii		29.4	53.9	5.1	11.6
C. lutea	0.4	29.4	37.7	11.1	21.4
C. epilobiifolia		0.3	19.6	67.9	12.2
C. stigulosa	0.9	18.3	13.8	45.2	21.8
Coconut	8	7	48	18	19

Table 2. Amino acid compositions of some potential oil seed species.

	Content (g/16 g of protein)			
Amino acids	*Cuphea painteri*	*Limnanthes douglasii* (Meadowfoam) VanEtten et al. 1961	*Lesquerella fendleri* Miller et al. 1962a	*Simmondsia chinensis* (Jojoba) Wisniak 1987
Aspartic acid	8.0	8.0	7.2	10.0

Threonine	3.0	4.3	4.5	6.0
Serine	4.9	4.4	4.6	6.9
Glutamic acid	15.3	16.3	13.7	10.4
Proline	3.5	4.2	6.7	6.0
Glycine	4.5	6.1	5.9	16.2
Alanine	3.9	4.4	4.5	6.3
Valine	4.6	5.0	4.8	5.4
Cystine	1.2	1.4	1.8	3.5
Methionine	1.7	1.4	1.3	0.9
Isoleucine	3.9	3.8	3.6	3.1
Leucine	6.2	6.9	5.8	6.8
Tyrosine	2.7	2.2	3.0	3.6
Phenylalanine	3.8	3.7	3.8	3.3
Lysine	3.9	6.9	6.6	4.3
Histidine	2.2	2.2	2.5	1.7
Arginine	10.2	7.5	7.9	5.6

Table 3. Seed characteristics of *Limnanthes alba.*

Seed characteristic	Content
Oil (%)	17-29
$20{:}1^{5}$ (%)	50-65
$22{:}1^{5} + 22{:}1^{13}$ (%)	10-29
$22{:}2^{5,13}$ (%)	15-30
Protein (%)	11-28
Glucosinolates (%)	3-10
Seed wt (g/1000)	4.2-9.8

Table 4. Fatty acid composition of *Lesquerella* spp.

	Content (% of total fatty acids)										
Species	palmitic 16:0	palmitoleic 16:1	stearic 18:0	oleic 18:1	linoleic 18:2	linolenic 18:3	ricinoleic 18:1-OH	densipolic 18:2-OH	lesquerolic 20:1-OH	auricolic 20:2-OH	Others
L. lindheimeri	1.5	0.5	1.5	11.8	5.8	0.9	0.9	tr	72.6	tr	4.0
L. fendleri	1.5	1.4	2.4	15.2	7.6	13.1	0.3	0.2	53.2	3.8	0.0
L. densipilia	5.8	1.2	2.6	22.1	3.0	10.1	2.0	50.7			1.3
L. auriculata	5.8	1.4	5.4	27.0	3.0	6.9	5.3	2.1	9.8	32.0	1.9

Table 5. Typical jojoba oil composition.

Chainlength	Fatty acids (%)	Alcohols (%)
16	2	1

18	13	1
20	71	51
22	13	42
24	1	5

Table 6. Fatty acid composition of epoxy oils.

	Content (% of total fatty acids)		
Fatty acids	*Vernonia galamensis*	*Stokesia laevis*	*Euphorbia lagascae*
16:0 (palmitic)	2.8	2.6	4.1
18:0 (stearic)	2.6	1.1	2.0
18:1 (oleic)	4.0	6.9	20.7
18:2 (linoleic)	12.8	13.7	9.2
18:3 (linolenic)	0.1	0.2	0.4
Vernolic acid	76.4	74.1	61.8
Others	1.3	1.4	1.8

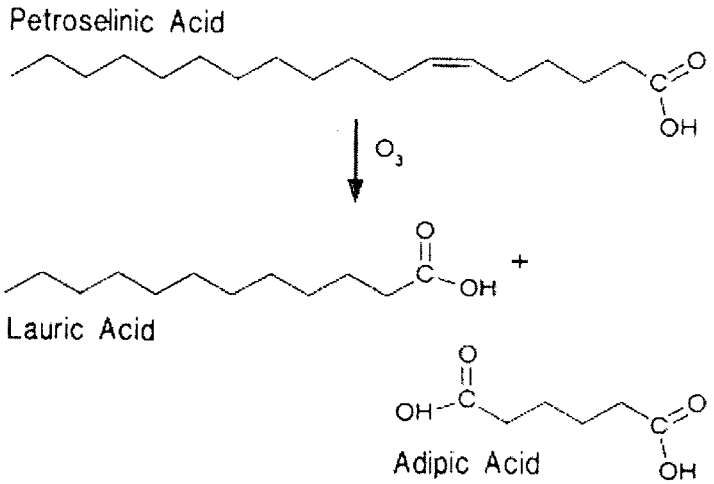

Fig. 1. Ozonolysis of petroselinic acid.

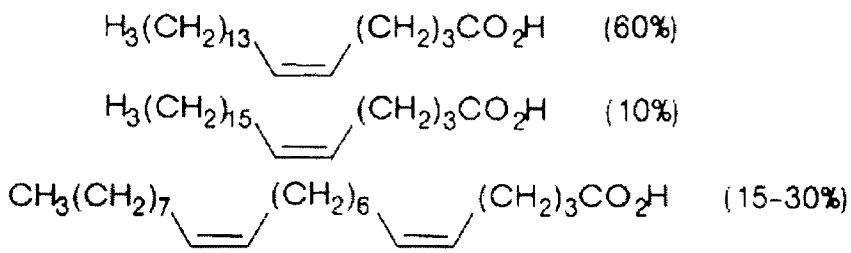

Fig. 2. Delta-5 acids of meadowfoam (*Limnanthes*) seed oil

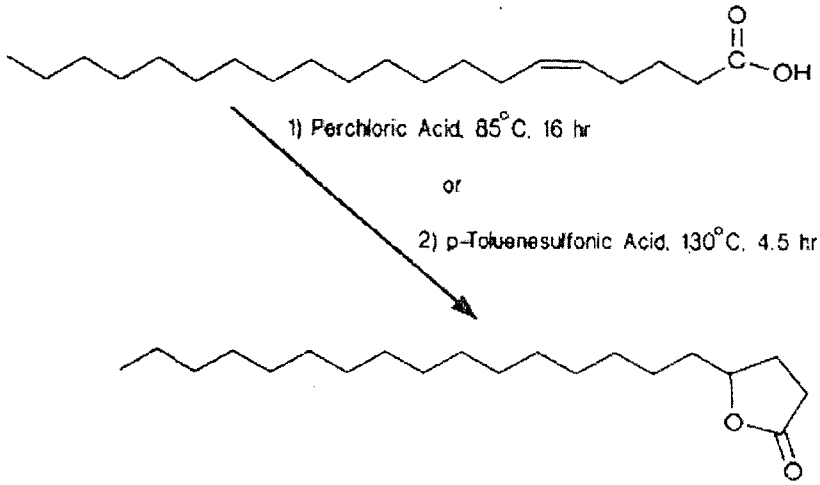

Fig. 3. Production of 4-eicosanolactone.

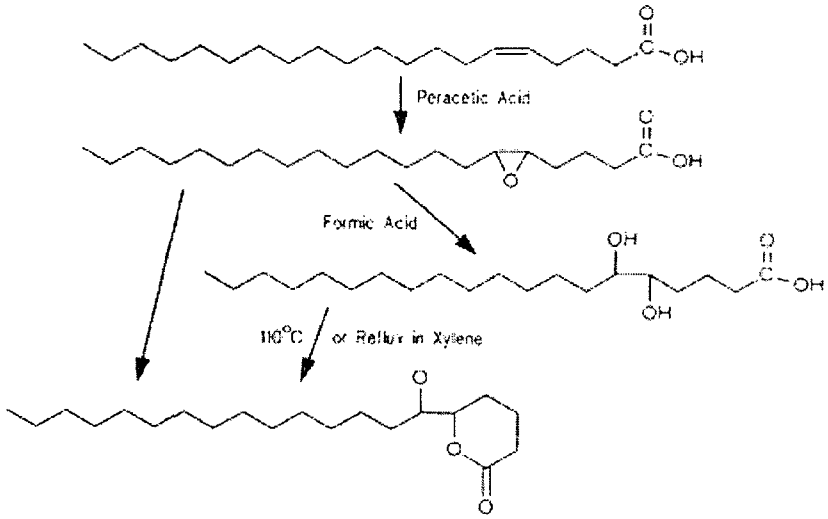

Fig. 4. Production of 6-hydroxy-5-eicosanolactone.

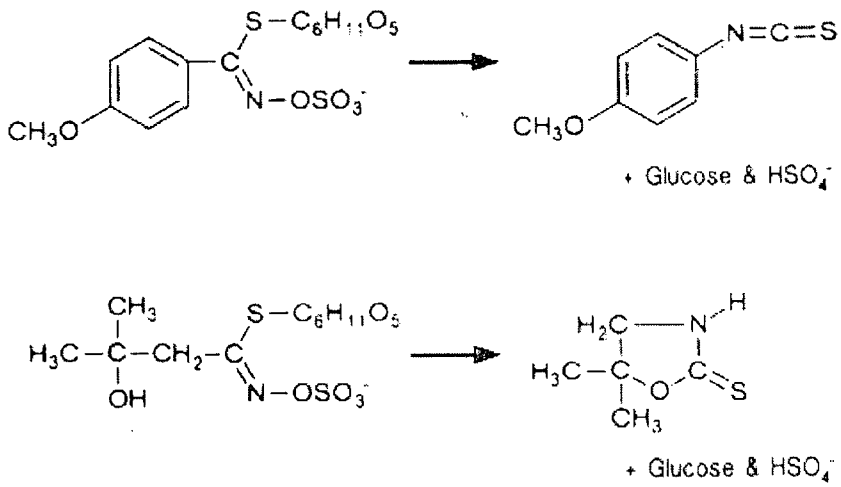

Fig. 5. Reaction of glucosinolates from meadowfoam seed.

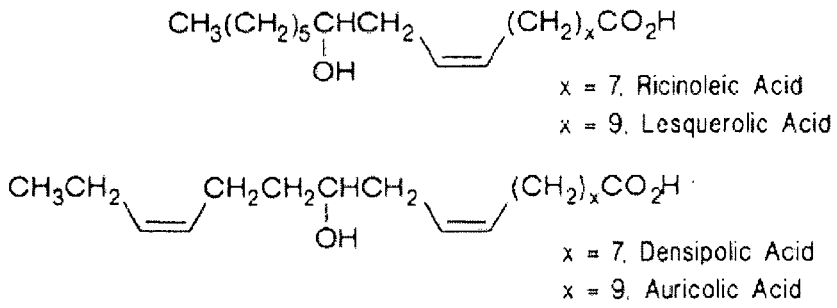

Fig. 6. Hydroxy acids from *Lesquerella* species.

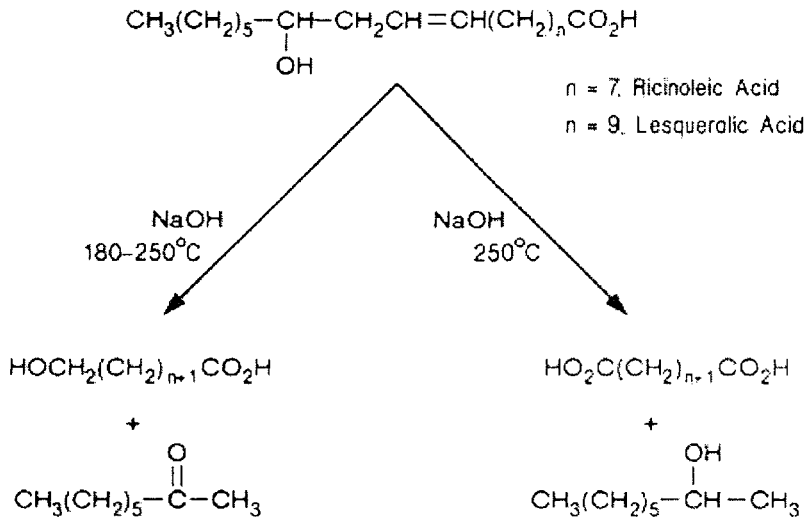

Fig. 7. Thermal-alkali reactions of hydroxy-monoene fatty acids.

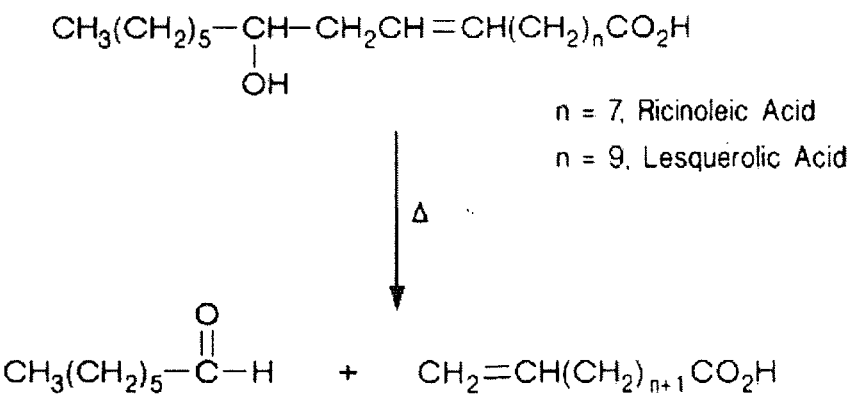

Fig. 8. Thermal reaction of hydroxy-monoene fatty acids.

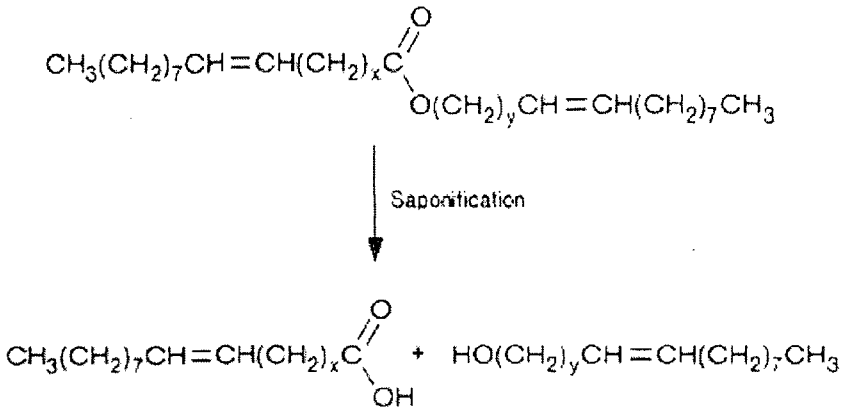

Fig. 9. Hydrolysis of jojoba wax-esters.

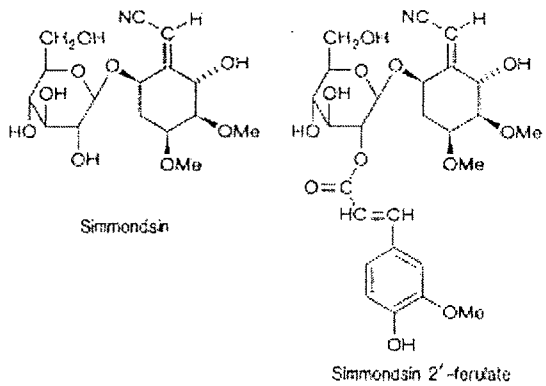

Fig. 10. Antinutritional compounds from jojoba seed.

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REFERENCE 9

ON THE FATTY ACIDS ESSENTIAL IN NUTRITION. III*

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After it was demonstrated (1) that a deficiency disease was caused by the lack of fatty acids in the diet, a study of the well known natural fatty acids was undertaken. In the second paper of this series (2) it was shown that none of the saturated fatty acids occurring in hydrogenated coconut oil was effective in curing the disease and promoting renewed growth of the animal. Pure methyl linolate is highly effective in curing sick animals and all oils which contain appreciable amounts of this acid are likewise good.

Since butter (30 per cent oleic acid) gave very poor results at the high level of 300 mg. daily (3 per cent of the diet) it was postulated that oleic acid is entirely negative and the small effects due to butter were due to traces of linoleic acid. But a sample of commercial methyl oleate gave good results and the value of oleic acid was left uncertain. Other common fatty acids which are now being studied are linolenic, arachidonic, and eleostearic. It is the object of this paper to report the results of some studies of these acids.

Diet and Technique

The constant temperature room is now maintained at 26.0° ± 1° the year round. The same cages and diets described in the first paper (1) are used for all work unless specifically stated to be changed. The maintenance diet, Diet 550-B, contains 12 per cent pure casein, 84.1 per cent sucrose, and 3.9 per cent salt mixture

* This work was supported by grants from the Medical Research Fund of the University of Minnesota, the National Research Council, and the Institute of American Meat Packers. Reported before the American Society of Biological Chemists at Montreal, 1931.

(McCollum Salt Mixture 185) (3). This is supplemented daily with 0.65 gm. of ether-extracted Northwestern dry yeast, and the non-saponifiable matter from 70 mg. of highest grade cod liver oil (Patch) and from 35 mg. of wheat germ oil. All known vitamins seem to be supplied in excess. The drinking water is distilled and contains 0.27 mg. of KI per liter.

Rats are weaned when 21 days old and put on the low fat diet. They must weigh over 36 gm. on weaning day. The weight curves reach a plateau when the rats are about 150 gm. in weight and when it has been established that they have reached their maximum weight and are actually declining slightly they are used as cures. Positive results are marked by a clearing of the skin, improvement of hair coat, and renewed growth both in length and weight. Increase in weight is used as the quantitative measure of the effectiveness of an oil or fatty acid.

EXPERIMENTAL

Linoleic Acid—In the preceding paper (2) 5 drops daily of methyl linolate were used. When larger or smaller doses are used (10 or 3 drops) marked differences of rate of response can be seen (Chart I). By similar studies on oils it has been demonstrated that maximum effects are reached at the 10 drop level. The pure methyl linolate was prepared from corn oil by the method of Rollett (4).

Oleic Acid—The preparation of oleic acid free from appreciable quantities of contaminating acids presents some difficulties. The method of Lapworth *et al.* (5) was used for olive oil. This procedure requires the separation of lead salts, preparation and purification of barium oleate, and finally esterification and distillation. This preparation is presumably free from linoleic acid and contains about 2 per cent palmitic acid, which does not interfere with our tests.

Since butter gave almost negative results as a curative fat, oleic acid was also prepared from it. Melted and filtered butter was saponified and esterified in the usual way and the esters subjected to fractional distillation. All of the lower fatty acid esters were removed at 140° and 3 mm. pressure. The residue was saponified and the oleic acid was purified by the same technique as used for olive oil. Yield, 75 cc. of methyl oleate from 800 gm. of butter.

The methyl oleate was fed to six rats, three receiving the olive oil acid and three the butter acid. The curves given in Chart II show that no growth resulted from the feeding of these esters over a period of 50 days. The hair coat, skin, and tail showed no improvement. There is no evidence that oleic acid has any curative effect although it may arrest the downward trend of the animals. It seems, therefore, that the slight positive effects noted

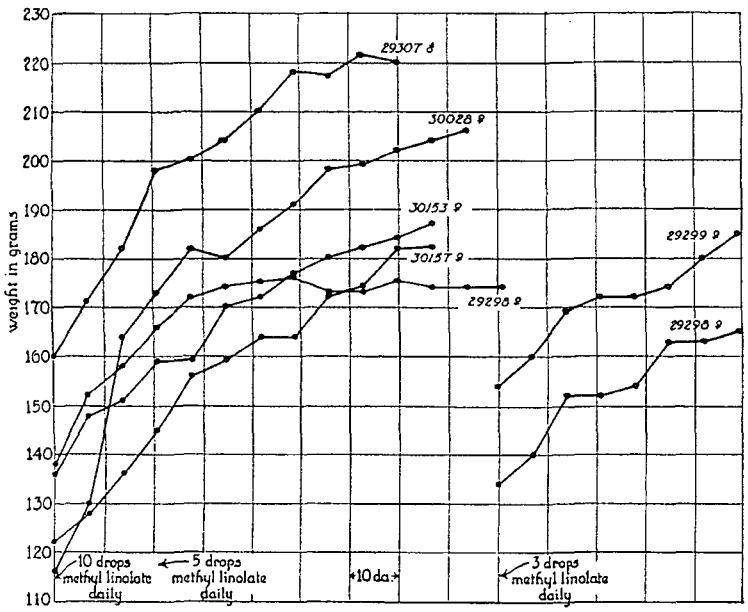

CHART I. Weight curves showing the growth response of rats to different quantities of methyl linolate. Along with the renewed growth there is rapid clearing of the skin.

for butter (2) are due to acids more unsaturated than oleic, probably linoleic.

Linolenic Acid—Although linolenic acid is usually absent from stored fats such as lard and tallow, it may be deposited in the fat depots if the food supply furnishes much of the acid (6). Levene and Rolf (7) have shown that in liver lecithin linolenic acid exceeds linoleic acid. However, Turner (8) found no linolenic acid in sheep liver.

Pure methyl linolenate was prepared from linseed oil by the method of Rollett (9). The hexabromide was recrystallized until it melted at 180–181° (uncorrected). This assured the elimination of all but traces of other fatty acids and their bromides. The hexabromide was then debrominated, esterified, and distilled at less than 1 mm. The water-clear ester was stored *in vacuo* until used. Special precautions were taken to prevent oxidation after each sealed tube was opened. The methyl linolenate was fed at a low level only. Results with three rats are given in Chart

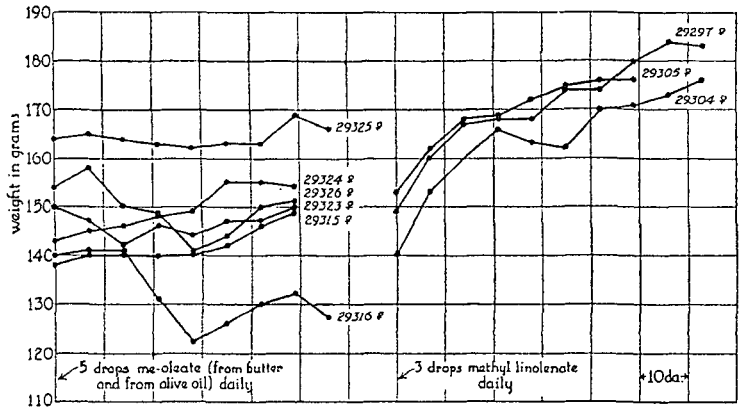

CHART II. Weight curves showing the growth response of rats to methyl oleate and methyl linolenate. Rats receiving methyl oleate show no renewed growth or clearing of the skin. Methyl linolenate quickly cleared the skin and the growth rate equaled that due to a like quantity of methyl linolate.

II. The gain in weight is almost identical with that for 3 drops of methyl linolate and the skin clears with great rapidity. It seems that linolenic acid can replace linoleic acid completely in the curing of rats suffering from a deficiency of fat.

α-Eleostearic Acid—Tung (China wood) oil is not ordinarily considered edible, but it has no harmful effects on rats when fed in small quantities. It is composed largely of the glyceride of α-eleostearic acid. A small amount of other unsaturated acids is present but linoleic and linolenic acids have not been reported present. α-Eleostearic acid melts at 48° and may be readily

changed into the β acid which melts at 71°. Since they readily absorb only 2 molecules of bromine the eleostearic acids were formerly considered isomeric with linoleic acid. The recent work of Böeseken and coworkers (10) shows that there are three double bonds in the eleostearic acids and that they are isomeric with linolenic acid. α-Eleostearic acid and its glycerides absorb oxygen very rapidly from the air and it is of interest to know whether this

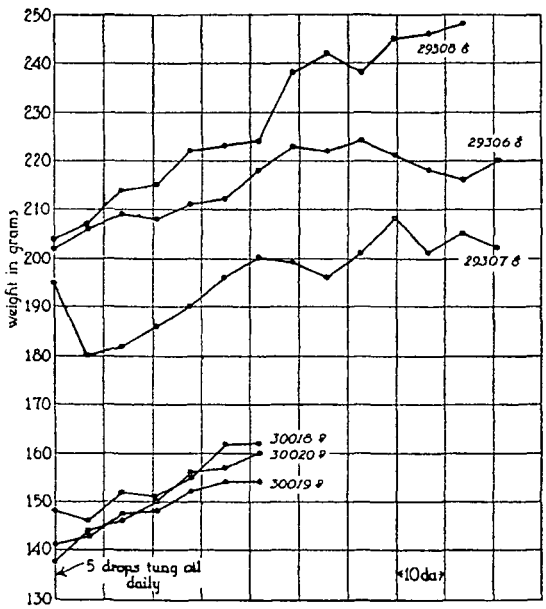

CHART III. Weight curves showing the slow response of rats to tung oil. The skin cleared very slowly.

acid which does not occur in animals can replace the more common linolenic acid. Tung oil was first fed to six rats. Slow but positive cures were effected. The skin gradually improved. The very gradual growth (Chart III) indicated that a trace of impurity rather than α-eleostearic acid was causing the response.

Pure α-eleostearic acid, m.p. 44–45°, was prepared from Florida tung oil.[1] This was given to rats in 5 drops doses for 2 weeks, but

[1] This oil was kindly furnished by Dr. J. S. Long, Lehigh University. It was the 1928 crop and had been kept under nitrogen.

they did not eat it well and the experiment was of little value. No tendency toward improvement was seen (Chart IV).

Methyl-α-eleostearate was then prepared by two methods. According to the first the acid was dissolved in an equal volume of absolute methyl alcohol and enough methyl alcoholic hydrogen chloride added to make a 4 per cent solution of HCl. This was left under CO_2 at room temperature overnight. The ester was purified by washing with dilute Na_2CO_3, distilled water, and $CaCl_2$ brine. The ester was finally taken up in ether, dried over anhydrous Na_2SO_4, and finally recovered *in vacuo*.

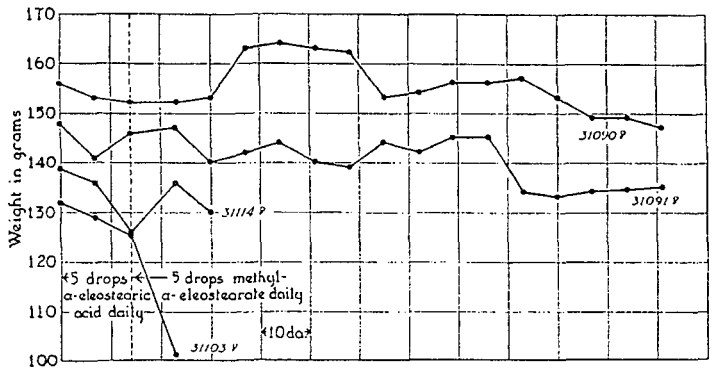

CHART IV. Weight curves showing the response of rats to α-eleostearic acid and to methyl-α-eleostearate. This acid fails to improve the skin or increase the weight.

This preparation is of high quality without distillation. Distillation must be avoided since it causes a rearrangement into the isomeric form, methyl-β-eleostearate. The α-eleostearate was sealed *in vacuo* and fed by dropping from a syringe so that the air was always excluded.

The other preparation was made by the very mild reagent, diazomethane.[2] The reaction goes smoothly and completely to give a high quality product requiring little or no purification.

Since there is some confusion in the literature concerning the

[2] We are indebted to Professor Lee I. Smith of the Department of Organic Chemistry for the first preparation made and for the detailed technique used by us.

rearrangement of α-eleostearic acid into β-eleostearic acid we checked our esters by saponifying small samples and recovering the free acid. After a single crystallization from alcohol the melting point was always between 43–45°. But when the methyl ester was distilled at 5 mm. pressure and the distillate was saponified, the β acid was recovered. After a single crystallization from alcohol it melted at 67–68°.

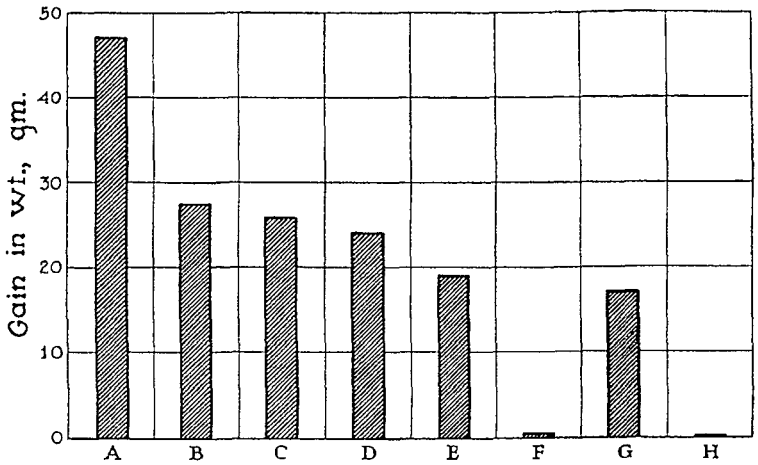

CHART V. A summary of the results given in the previous charts. The columns give the average maximum gains in weight of groups receiving the supplements over a period of 40 days. Column A, 7½ drops of methyl linolate daily; Column B, 3 drops of methyl linolate daily; Column C, 3 drops of methyl linolenate daily; Column D, 3 drops of methyl linolate plus methyl linolenate (1:1 mixture) daily; Column E, 3 drops of methyl linolate plus methyl linolenate (1:1 mixture) plus 10 per cent methyl arachidonate daily; Column F, 5 drops of methyl oleate (from butter and from olive oil) daily; Column G, 5 drops of tung oil (90 per cent eleostearic acid) daily; Column H, 5 drops of methyl-α-eleostearate daily.

All of the preparations gave the same nutritional results. Some curves are shown in Chart IV. There is no evidence of improvement, either in weight or in skin condition.

DISCUSSION

A general summation of the comparisons of oleic, linoleic, linolenic, and α-eleostearic acids is given in Chart V. In all cases

where considerable growth took place the skin cleared and the rats were generally improved. A better muscle tone is always noticeable after a rat has been cured.

By this work oleic acid has been definitely grouped with the saturated acids as ineffective in the curing of rats subnormal because of the lack of fat. This substantiates the arguments put forth in the second paper of this series (2) that it is possible for animals to synthesize from carbohydrates large amounts of fat and still suffer from a fat deficiency. The review of the literature will not be repeated here but it seems clear that warm blooded animals synthesize only the saturated acids and oleic acid and that they are dependent upon the food supply for linoleic and linolenic acids. One of these two acids must be ingested by the rat if it is to survive and our findings indicate that they are interchangeable in the tissues. Further work is being done on the relative values of the two.

The comparison of whole tung oil with methyl-α-eleostearate is interesting. Since the α-eleostearic acid does not have any curative effect it is evident that there is an acid in tung oil in small amounts which causes the renewed growth. Similar effects were seen when 15 drops of butter were fed daily to rats (2). Since pure oleic acid and the saturated fatty acids are ineffective, small amounts of undetermined acids are assumed to be present. These acids are probably linoleic or linolenic.

A mixture of linoleic and linolenic esters is of no more value than either of the esters alone (Chart V, Column D). This is interesting since tissues normally have a mixture of the two. When methyl arachidonate was added as 10 per cent of the mixture the animals uniformly showed less response (Chart V, Column E). The reason for this is not at all clear. Lard contains appreciable amounts of arachidonic acid and it is one of the best curative fats. Liver and liver fat are rich sources of arachidonic acid. Both have been used by us as preventives for the fat deficiency and have proved highly effective. Since there is no reason to attribute toxic effects to small amounts of arachidonic acid it seems probable that some of the purified arachidonic acid which we have fed has been altered in the process of preparation.

CONCLUSIONS

1. Both linolenic acid and linoleic acid are effective in curing rats suffering from a fat deficiency. They seem to be about equal in value and can replace each other in the tissues.

2. Oleic acid is ineffective in the curing of sick rats and is classed with the saturated acids.

3. α-Eleostearic acid, an isomer of linolenic acid, is ineffective in curing sick rats. This might be attributed to its high melting point.

4. Tung oil, like butter, has enough undetermined unsaturated acids to effect slow cures.

5. Mixtures of linoleic and linolenic esters are no more effective than a single ester, while the addition of a preparation of methyl arachidonate has a slight unexplained depressing effect.

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REFERENCE 10

gestation) of 0 or 1500 mg/kg/day nonanoic acid in a corn oil vehicle. Dams did not show excess mortality, effects on body weight or food consumption, or other clinical signs. No lesions were noted in dams at necropsy. Pregnancy rates, corpora lutea, implantations, implantation efficiency, and uterine and ovarian weights were comparable to controls. Pups were delivered on day 20 of gestation by Caesarian section to determine feto-embryo toxicity and developmental effects. No excess resorptions, decreased pup weights or body lengths, decreased viability, or decreased litter size were noted. No statistically significant difference was detected in skeletal or soft tissue malformations, although two cleft palates, two instances of small tongue, and one instance of hydroureter occurred in the treated group (of 80 treated pups from 22 litters), whereas none occurred in the controls (1).

15.4.1.5 Carcinogenesis. No information was found in the literature.

15.4.1.6 Genetic and Related Cellular Effects Studies. No information was found in the literature.

15.4.2 Human Experience

A 12% solution of nonanoic acid in petrolatum produced no irritation on human skin after a 48 h closed insult patch test (43). Higher concentrations (0.5 or 1.0 M in propanol) caused irritation when applied under occlusive patches. No sensitization reactions were produced in 25 volunteers after patch testing with nonanoic acid (12% in petrolatum) (43).

Various concentrations of nonanoic acid in 1-propanol were applied to 116 healthy volunteers and 75 dermatitis patients. A concentration of 20% produced skin reactions in 90 to 94% of subjects. Effects consisted of erythema at 48 h and pigmentation at 96 h (247). Human volunteers patch tested with nonanoic acid showed morphological changes in the exposed skin indicating disturbances in keratinocyte metabolism and differentiation, giving rise to dyskeratosis and parakeratosis, respectively (248).

15.5 Standards Regulations or Guidelines of Exposure

Nonanoic acid is generally recognized as safe (GRAS) for use as a synthetic flavoring substance (21 CFR 172.515) and was approved by the Council of Europe (1974) for food use (43).

16.0 Capric Acid

16.0.1 CAS Number: *[334-48-5]*

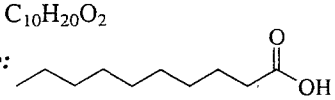
16.0.2 Synonyms: Caprinic acid; decanoic acid;, decylic acid; decoic acid; 1-nonanecarboxylic acid

16.0.3 Trade Names: NA

16.0.4 Molecular Weight: 172.27

16.0.5 Molecular Formula: $C_{10}H_{20}O_2$

16.0.6 Molecular Structure:



16.1 Chemical and Physical Properties

See Table 70.1.

16.2 Production and Use

Capric acid is chiefly used in the production of esters for perfumes and fruit flavorings, as a base for wetting agents, and in the production of intermediates, plasticizers, resins, and food additives (2).

16.3 Exposure Assessment

16.3.3 Workplace Methods: NA

16.4 Toxic Effects

16.4.1 Experimental Studies

16.4.1.1 Acute Toxicity. The acute toxicity of capric acid is summarized in Table 70.2. The compound was a moderate to severe irritant when applied undiluted for 24 h to intact or abraded rabbit skin in an occluded patch test (48).

According to Smyth et al. (17), capric acid (mixed isomers) produces severe corneal burns when applied as a 5% solution (0.5 mL in water or propylene glycol) to rabbit eyes and was moderately irritating to rabbit skin in an open patch test. No deaths occurred in rats exposed for 8 h to concentrated capric acid vapor (17).

16.4.1.2 Chronic and Subchronic Toxicity. No gastric lesions were evident in rats fed capric acid (10% in diet) for 150 days (250). Capric acid administered daily (37 mg/kg) to pregnant rabbits increased sensitivity to oxytocin-induced labor (48).

16.4.1.3 Pharmacokinetics, Metabolism, and Mechanisms. Capric acid is metabolized by the β-oxidative pathway and gives rise to C_8 and C_6 dicarboxylic acids (suberic and adipic acids) in rats (251). Capric acid metabolism also produced ketone bodies in rats (252), rabbits (253), dogs (83), piglets (254), and goats (255). Activation of lipid metabolism by starvation, fat-feeding, and experimental diabetes increased the extent of ketosis in rats (251). ω-Oxidation that led to the excretion of sebacic acid (255) and chain elongation reactions (253–255) has been reported. Metabolism of capric acid is rapid; in humans given[1-^{14}C]decanoic acid orally, about 52% of the radioactivity was recovered within 2.5 to 4 h (260).

16.4.1.4 Reproductive and Developmental. When fed to rats over three successive generations as the triglyceride (in combination with octanoic acid triglyceride), increased mortality was detected in the third generation, attributed by the authors to decreased nutritional content of the second-generation mothers milk (257).

16.4.1.5 Carcinogenesis. No evidence of carcinogenicity was reported in limited oral studies of rats (257).

16.4.1.6 Genetic and Related Cel
the Ames *Salmonella* assay (257). (
against a number of bacterial s
citrovorum (259).

16.4.2 Human Experience

Capric acid produced no irritation
48 h in a closed patch test (48). A
compound produced signs of irrit
volunteers (260). No sensitization

16.5 Standards, Regulations or

Capric acid is generally recognized
was approved by the Council of
approved by the U.S. Food and
applications and in the dairy indus

17.0 Undecylic Acid

17.0.1 CAS Number: [112-37-8

17.0.2 Synonyms: *n*-Undecanoi
carboxylic acid

17.0.3 Trade Names: NA

17.0.4 Molecular Weight: 186.

17.0.5 Molecular Formula: C_{11}

17.0.6 Molecular Structure:

17.1 Chemical and Physical Pr

See Table 70.1.

17.2 Production and Use

The chief use of this acid is in che

17.3 Exposure Assessment

17.3.3 Workplace Methods: N

16.4.1.6 Genetic and Related Cellular Effects Studies. Decanoic acid was negative in the Ames *Salmonella* assay (257). Capric acid is highly effective as an antimicrobial agent against a number of bacterial strains, including *B. subtilis* (258) and *Leuconostoc citrovorum* (259).

16.4.2 Human Experience

Capric acid produced no irritation when 1% in petrolatum was applied to human skin for 48 h in a closed patch test (48). At higher concentrations (up to 1.0 M in propanol), the compound produced signs of irritation within 8 days in occlusive patch tests in human volunteers (260). No sensitization reactions were seen.

16.5 Standards, Regulations or Guidelines of Exposure

Capric acid is generally recognized as safe for use as a food additive(21 CFR 182.60) and was approved by the Council of Europe (1974) for food use (157). The acid is also approved by the U.S. Food and Drug Administration for use as a sanitizer for food applications and in the dairy industry (261, 262).

17.0 Undecylic Acid

17.0.1 CAS Number: *[112-37-8]*

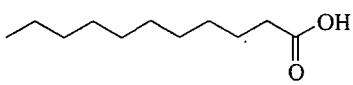
17.0.2 Synonyms: *n*-Undecanoic acid; *n*-undecoic acid; hendecanoic acid; 1-decanecarboxylic acid

17.0.3 Trade Names: NA

17.0.4 Molecular Weight: 186.29

17.0.5 Molecular Formula: $C_{11}H_{22}O_2$

17.0.6 Molecular Structure:



17.1 Chemical and Physical Properties

See Table 70.1.

17.2 Production and Use

The chief use of this acid is in chemical syntheses.

17.3 Exposure Assessment

17.3.3 Workplace Methods: NA

REFERENCE 11

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- **Edible Oil Processing**
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- **The history of lipid science and technology**
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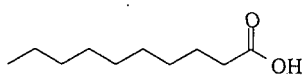
REFERENCE 12

Salmo gairdneri	96 h NOEC	1 mg/L	
Catostomus commersoni	96 h NOEC	1 mg/L	
Ictalurus melas	96 h NOEC	1 mg/L	
Lepomis cyanellus	96 h NOEC	1 mg/L	
Perca flavescens	96 h NOEC	1 mg/L	(7789)
Petromyzon marinus	24 h EC_0	5 mg/L	(7790)

n-capric acid (decanoic acid; *n*-decoic acid; *n*-decylic acid)



$CH_3(CH_2)_8COOH$

$C_{10}H_{20}O_2$

CAS 334-48-5

USES: esters for perfumes and fruit flavors, base for wetting agents; intermediate; plasticizer.

A. PROPERTIES: molecular weight 172.26; melting point 31.5°C; boiling point 268–270°C; vapor pressure 1 mm at 125°C; sp. gr. 0.89 at 40/4°C; log P_{oct} 1.88.

B. AIR POLLUTION FACTORS:

Odor: threshold odor conc.

0.014 mg/m^3 = 1.96 ppb	(307)
detection 0.05 mg/m^3	(778, 779)
recognition 0.08–0.09 mg/m^3	(610)

C. WATER POLLUTION FACTORS:

BOD_5:	9% of ThOD
COD:	85% of ThOD
Waste water treatment:	
A.S.: after	6 h: 11% of ThOD
	12 h: 19% of ThOD
	24 h: 23% of ThOD (89)

Odor threshold: detection: 10 mg/kg. (886)

D. BIOLOGICAL EFFECTS:

FISHES:

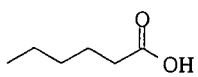
Lepomis macrochirus: chemical is too insoluble to be toxic (1294)

Red killifish *(Oryzias latipes):* 96h LC_{50}: in seawater: 31 mg/L; in freshwater: 20 mg/L; 54 mg/L (sodium caprate) (2631)

Gammarus *(Hyale plumulosa):* 96h LC_{50}: 41 mg/L (2631)

caproaldehyde *see* *n*-hexaldehyde

caproic acid (hexanoic acid; *n*-hexoic acid)



$CH_3(CH_2)_4COOH$

REFERENCE 13

13

DECANOIC ACID

CASRN: 334-48-5

For other data, click on the Table of Contents

Environmental Biodegradation:

AEROBIC: The 5 day BOD of **decanoic acid,** concn 100 ppm, was determined to be 8.52 mmol/mmol **decanoic acid** using acclimated mixed microbial cultures in a mineral salt medium (1). **Decanoic acid,** present at 10,000 ppm, reached 45 to 53% and 46 to 54% of its theoretical BOD in 5 and 20 days, respectively, using a sewage inoculum(2). **Decanoic acid,** present at 10,000 ppm, reached 13, 45, and 46% of its theoretical BOD in 5, 10, and 20 days, respectively, using a sewage inoculum(3). In a similar study, **decanoic acid,** present at 10,000 ppm, reached 49, 53, and 54% of its theoretical BOD in 5, 10, and 20 days, respectively, using an acclimated sewage inoculum(3). **Decanoic acid,** present at unknown concn, reached 9% of its theoretical BOD in 5 days using a sewage inoculum(4). Using the Warburg test method, **decanoic acid,** present at 500 ppm, reached 29 to 42% of its theoretical BOD in 1 day, using an activated sludge inoculum with a microbial population of 2,500 mg/L corrected for endogenous respiration(5). Biodegradation of 100 ppm **decanoic acid** using the cultivation method was 100% in river water and 100% in sea water after 3 days(6). The theoretical oxygen demand for 500 mg/L **decanoic acid** was determined to be 10.9%, 18.9%, and 23.4% after 6, 12, and 24 hours of exposure to activated sludge solids at 2,500 mg/L in the Warburg respirometer(7). An aerobic biodegradation screening study of **decanoic acid,** based on BOD measurements, using a sewage inoculum and an unknown **decanoic acid** concn, indicated 23% of its theoretical BOD over a period of 20 days(8). The biodegradation of 100 mg/L **decanoic acid** by non-acclimated activated sludge over an unspecified time period was determined to have 100% total organic carbon removal(9).

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REFERENCE 14

14

INTERNATIONAL UNION OF
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SECTION OF ANALYTICAL CHEMISTRY
COMMISSION ON ELECTROCHEMICAL DATA

DISSOCIATION CONSTANTS OF ORGANIC ACIDS IN AQUEOUS SOLUTION

G. KORTÜM, W. VOGEL and K. ANDRUSSOW
Institute of Physical Chemistry
University of Tübingen

LONDON
BUTTERWORTHS
1961

INTERNATIO
REINE UND

ABTEILUNG F
KOMMISSION FÜ

DISSOZIA
ORGAN
IN WÄSS

G. KORTÜM, W
Institut
der

BUT

16	Önanthsäure: $CH_3 \cdot (CH_2)_5 \cdot COOH$					
	(*c*) $1{,}28 \cdot 10^{-5}$	25	$\varkappa = 1$ $\mathcal{J} = 0{,}002$ bis $0{,}005$	**L1** **R1a** **K2e**	rel. zuv. Wie bei iso-Buttersäure (D9)	D9
	(*c*) $1{,}42 \cdot 10^{-5}$	18	$\varkappa = 2$ $\mathcal{J} = 0{,}01$ bis $0{,}03$	**E2b**	unsicher Die verwendete Säure hatte ein Äquivalentgewicht von 131,5 (theoret. 130,1).	L13
17	tert.-Amyl-Essigsäure: $(CH_3)_3C \cdot (CH_2)_2 \cdot COOH$					
	(*c*) $1{,}63 \cdot 10^{-5}$	18	$\varkappa = 0{,}3$ $\mathcal{J} = 0{,}01$ bis $0{,}03$	**E2b**	zuv.	L7

18	Äthyl-n-Propyl-Essigsäure: $CH_3 \cdot (CH_2)_2 \cdot CH(CH_2 \cdot CH_3) \cdot COOH$					
	(*c*) $1{,}96 \cdot 10^{-5}$	18	$\varkappa = 0{,}3$ $\mathcal{J} = 0{,}01$ bis 3	**E2b**	zuv.	L7
19	Caprylsäure: $CH_3 \cdot (CH_2)_6 \cdot COOH$					
	(*c*) $1{,}275 \cdot 10^{-5}$	25	$\varkappa = 1$ $\mathcal{J} = 0{,}0005$ bis $0{,}015$	**L1** **R1a** **K2e**	rel. zuv. Wie bei iso-Buttersäure (D9)	D9
20	Pelargonsäure: $CH_3 \cdot (CH_2)_7 \cdot COOH$					
	(*c*) $1{,}11 \cdot 10^{-5}$	25	$\varkappa = 1$ $\mathcal{J} = 0{,}0004$ bis $0{,}001$	**L1** **R1a** **K2e**	rel. zuv. Wie bei iso-Buttersäure (D9)	D9
21	Oxalsäure: COOH·COOH					
	(*m*) K_2 $5{,}91 \cdot 10^{-5}$	0	$\varkappa = 1$ $\mathcal{J} = 0{,}025$ bis $0{,}16$	**E1a**	rel. zuv. Ungenaue Korr. für weitere Dissoziation des Bioxalations (vgl. P7).	H17
	5,82	5				
	5,70	10				
	5,55	15				
	5,40	20				
	5,18	25				
	4,92	30				
	4,67	35				
	4,41	40				
	4,09	45				
	3,83	50				
	(*c*) K_1 $5{,}36 \cdot 10^{-2}$ K_2 $5{,}3 \cdot 10^{-5}$	25	$\mathcal{J} = 0{,}0002$ bis $0{,}03$	**L1** **R1b**	K_1 rel. zuv. K_2 unsicher. Angewendetes Rechenverfahren führt per Definition zu konstantem K_1; daß auch für K_2 konstanter Wert erzielt wird, wird als Beweis für die Richtigkeit der Methode angesehen. Anwendung der D.H.-Gl. u. der Shedlovsky'schen für Λ_c bei den höheren Konzentrationen bedingt Unsicherheiten. Andrerseits stützt sich die Berechnung von K_1 gerade auf diese Konz.-Gebiete. K_2 wird als Korrekturgröße berechnet.	D1

REFERENCE 15

as opposed to the trend in t-butyl
that water+water interactions are
gen bonds are being formed. The
utyl alcohol system, the former does
d formation between methyl cyanide

y to the behaviour of water which
There is, therefore, a trend to endo-
structure. One way for the system
separate into two phases. Since the
with decrease in temperature, the
ease and occurs below −1°. This
in the sound absorption peak in the
critical solution temperature. With
und absorption increases markedly
sound absorption P.S.A.C., for the
sely with the minimum in the partial
ncy towards immiscibility increases
haracteristics are dominated by the
ecules.

onsored by the U.S. Department of
We also thank the Science Research
and Huntingdon and Peterborough
M. J. W.

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Heats of Sublimation and Vaporization at 25° of Long-Chain Fatty Acids and Methyl Esters[1]

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The vapour pressures of nonanoic (20-30°), decanoic (20-30°), undecanoic (20-35°), and dodecanoic (20-35°) acids have been measured using a modified Knudsen effusion method. The vapour pressure of methyl tridecanoate (22·3°, 26°) and methyl pentadecanoate (22-30°) have also been measured using the classical Knudsen method. Heats of sublimation and vaporization at 25° are calculated and compared to literature values for other fatty acids and methyl esters. Empirical equations are derived to predict the heats of sublimation and vaporization at 25° of fatty acids and methyl esters.

EXPERIMENTAL

The vapour pressures of liquid nonanoic (20-30°), solid decanoic (20-30°), solid and liquid undecanoic (20-35°), and solid dodecanoic (20-35)° acids have been determined using a modified Knudsen effusion method. The method utilizes a glass-wool plug saturated with tricresyl phosphate placed about 2 cm above the Pyrex effusion cell orifice.[3] The effusion cell was a Pyrex sphere, 1 cm diam. and weighing about 60 mg. The orifice area was determined by measuring the rate of effusion of benzophenone [4] and found to be 0·003771 cm^2 (±1 %). The value determined using a microscope was 0·0037 cm^2 (±3 %). The vapour pressures of liquid methyl tridecanoate at 22·34 and 25·95° and methyl pentadecanoate (22-30°) have also been measured using the classical Knudsen method.[5] The effusion cell was also a Pyrex sphere and had an orifice area of 0·006489 cm^2 (±0·4 %) as determined from the measurements of the rate of effusion of benzophenone.[4] The area determined using a microscope was 0·0067 cm^2 (±3 %).

All of the fatty acids and the methyl pentadecanoate were analyzed by GLC and found to contain less than 0·3 % homologue impurity. Powder X-ray data for the fatty acids showed that they crystallized in the *C* (or *C'*) form and remained in this form for the time intervals and at the temperatures of the effusion runs.[6]

In addition to the orifice area measurements using benzophenone, the rates of effusion of dodecanoic acid at 30 and 35° were measured using both the modified and classical Knudsen methods. At 35°, the agreement between the two methods was almost exact; at 30°, the classical method gave results about 3 % higher but well within the observed error of the two sets of results.

RESULTS AND CALCULATIONS

The results are given in table 1. The equation

$$P = 4RTQ/\bar{c}A, \qquad (1)$$

derived from kinetic theory, is used to calculate the vapour pressures from the effusion data. In this equation, P is the vapour pressure in mm Hg, R is $6{\cdot}2363 \times 10^4$ mm Hg cm^3/deg. mole, A is the orifice area determined experimentally, $\bar{c}$ is the mean velocity of the effusate molecules at temperature T, and Q is the rate of effusion expressed as mole/sec.

The heats of sublimation and vaporization were found from least squares plots of $\log_{10}$ (vapour pressure) against $1/T$. The errors in these values are the mean standard deviations in the slopes of the corresponding plots.

DISCUSSION

Vapour phase heats of formation at 25° are used in molecular structure studies. These values are often calculated from condensed phase heats of combustion and heats of sublimation or vaporization at 25°. For this reason, it is useful to know the heats of sublimation or vaporization at 25° of as many compounds as possible.

TABLE 1.—EFFUSION DATA AND RESULTS

acid or ester	temp. range, °C	number of determinations	least-squares eqn. (*a*)	ΔH_{sub} or ΔH_{vap} kcal/mole
nonanoic (liquid)	20-30	3	$\log_{10}P = -4305\,(1/T) + 11{\cdot}587$	19·7±0·1
decanoic (solid *C*-form)	20-30	3	$\log_{10}P = -6207\,(1/T) + 17{\cdot}382$	28·4±0·5
undecanoic (liquid)	30-35	3	$\log_{10}P = -5108\,(1/T) + 13{\cdot}291$	23·4±1·5
undecanoic (solid *C'*-form)	20-27	6	$\log_{10}P = -6348\,(1/T) + 17{\cdot}418$	29·0±0·3
dodecanoic (solid *C*-form)	20-35	4	$\log_{10}P = -6923\,(1/T) + 18{\cdot}531$	31·7±0·4
methyl tridecanoate (liquid)	22·34 25·95	2	$P = 8{\cdot}62\times10^{-4}$ $P = 1{\cdot}29\times10^{-3}$	19·5±1·0 [b]
methyl pentadecanoate (liquid)	22-30	4	$\log_{10}P = -4594\,(1/T) + 11{\cdot}620$	21·0±0·3

(*a*) P in mm Hg.
(*b*) This error is calculated from the deviations of the individual vapour pressure values.

Long-chain fatty acids and methyl esters have very low vapour pressures and, as chain lengths increase these vapour pressures become increasingly difficult to measure. At 25°, the vapour pressures of fatty acids up to dodecanoic acid [7, 8] and of methyl esters up to methyl hexadecanoate [9; 10] have been measured. Therefore, the heats of sublimation of these compounds at 25° are known using the Clausius Clapeyron equation. In this discussion the relations between chain length and temperature against heats of sublimation and vaporization are estimated from the data. These make it possible to calculate the heats of sublimation and vaporization at 25° for fatty acids and their methyl esters up to a chain length of 20 carbon atoms, excluding only the odd carbon-number solid methyl esters.

First, an equation is derived which relates chain length and the heats of vaporization and sublimation at 25°. Certain data [11, 12] for fatty acids enable heats of vaporization at certain temperatures to be calculated over a considerable range of chain lengths. The value 1·1±0·1 kcal/mole per CH_2 group at 25° can be estimated from these data as the increment in the heats of vaporization. This value is somewhat lower at higher temperatures (>100°). The increment in the heats of fusion of fatty acids calculated from the data of Adriaanse [13] is 1·0 kcal/mole per CH_2

group. Others [14] have found a
average value of 1·0±0·1 kcal/m
overall increment in the heats o
kcal/mole per CH_2 group making
ture dependent. For even carbo
of decanoic acid at 25° [8] is used
number acids, the undecanoic ac

$\Delta H_{sub,25°} = 28{\cdot}4+$

$\Delta H_{sub,25°} = 29{\cdot}0+$

The heat of vaporization of dec
fusion [13] from its heat of sublii
relating heats of vaporization of

$\Delta H_{vap,25°} = 2$

The increment in the heats of
to be 1·1 kcal/mole per CH_2 gro
is estimated at 1·0 kcal/mole pe
for methyl hexadecanoate at 2:
number methyl ester heats of su

$\Delta H_{sub,25°} = 37{\cdot}0+$

No data are available for solid
methyl tridecanoate from this
methyl esters as represented in e

$\Delta H_{vap,25°} = 19$

Values calculated using these eq
Second, a relationship betwee
For fatty acids,[11, 12] the decreas
data to be 3 %/10°, and for metl
ture corrections are also taken
sidering the temperature depend
estimated corrections are applie
results are shown in table 2.

The value for the heat of vap
data given by Stull [18] is 8·0 kc
kcal/mole. The difference is we
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In a similar way, the heat o
vapour pressure data [18] is 9·8
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ıding plots.

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ısed in molecular structure studies.
:d phase heats of combustion and
ır this reason, it is useful to know
as many compounds as possible.

ND RESULTS

least-squares eqn. (a)	ΔH_{sub} or ΔH_{vap} kcal/mole
$\log_{10}P = -4305\ (1/T) + 11{\cdot}587$	$19{\cdot}7 \pm 0{\cdot}1$
$\log_{10}P = -6207\ (1/T) + 17{\cdot}382$	$28{\cdot}4 \pm 0{\cdot}5$
$\log_{10}P = -5108\ (1/T) + 13{\cdot}291$	$23{\cdot}4 \pm 1{\cdot}5$
$\log_{10}P = -6348\ (1/T) + 17{\cdot}418$	$29{\cdot}0 \pm 0{\cdot}3$
$\log_{10}P = -6923\ (1/T) + 18{\cdot}531$	$31{\cdot}7 \pm 0{\cdot}4$
$P = 8{\cdot}62 \times 10^{-4}$ $P = 1{\cdot}29 \times 10^{-3}$	$19{\cdot}5 \pm 1{\cdot}0$ [b]
$\log_{10}P = -4594\ (1/T) + 11{\cdot}620$	$21{\cdot}0 \pm 0{\cdot}3$

ıdividual vapour pressure values.

very low vapour pressures and, as
ne increasingly difficult to measure.
dodecanoic acid [7, 8] and of methyl
n measured. Therefore, the heats
own using the Clausius Clapeyron
een chain length and temperature
e estimated from the data. These
ıation and vaporization at 25° for
ngth of 20 carbon atoms, excluding

n length and the heats of vaporiza-
[12] for fatty acids enable heats of
lated over a considerable range of
CH_2 group at 25° can be estimated
vaporization. This value is some-
ıe increment in the heats of fusion
aanse [13] is 1·0 kcal/mole per CH_2

group. Others [14] have found a value of 1·0 kcal/mole per CH_2 group also. The average value of 1·0±0·1 kcal/mole per CH_2 group is taken for this increment. The overall increment in the heats of sublimation at 25° is therefore estimated to be 2·1 kcal/mole per CH_2 group making the assumption that heats of fusion are not temperature dependent. For even carbon-number acids, the value of the heat of sublimation of decanoic acid at 25° [8] is used as the reference value in eqn. (2). For odd carbon-number acids, the undecanoic acid value found in this work is used.

$$\Delta H_{sub,25°} = 28{\cdot}4 + (n-10)(2{\cdot}1) \text{ even carbon-number acids,} \quad (2)$$

$$\Delta H_{sub,25°} = 29{\cdot}0 + (n-11)(2{\cdot}1) \text{ odd carbon-number acids.} \quad (3)$$

The heat of vaporization of decanoic acid at 25° is found by subtracting its heat of fusion [13] from its heat of sublimation and is used as a reference value in eqn. (4) relating heats of vaporization of fatty acids at 25° with chain length:

$$\Delta H_{vap,25°} = 21{\cdot}4 + (n-10)(1{\cdot}1) \text{ liquid fatty acids.} \quad (4)$$

The increment in the heats of vaporization for methyl esters can also be estimated to be 1·1 kcal/mole per CH_2 group [10, 15, 16] and the increment in the heats of fusion is estimated at 1·0 kcal/mole per CH_2 group.[13, 17] The Davies and Kybett value for methyl hexadecanoate at 25° [9] is used as a reference value for even carbon-number methyl ester heats of sublimation in eqn. (5).

$$\Delta H_{sub,25°} = 37{\cdot}0 + (n-16)(2{\cdot}1) \text{ even carbon-number esters.} \quad (5)$$

No data are available for solid odd carbon-number esters. The value for liquid methyl tridecanoate from this work is used for the reference value for the liquid methyl esters as represented in eqn. (6):

$$\Delta H_{vap,25°} = 19{\cdot}5 + (n-13)(1{\cdot}1) \text{ liquid methyl esters.} \quad (6)$$

Values calculated using these equations are shown in table 2.

Second, a relationship between heats of sublimation and temperature is developed. For fatty acids,[11, 12] the decrease in the heats of vaporization is estimated from these data to be 3 %/10°, and for methyl esters [10, 15, 16] to be 1·5 %/10°. These temperature corrections are also taken for the decrement in the heats of sublimation, considering the temperature dependence of the heats of fusion to be negligible. These estimated corrections are applied to the data in ref. (7), (9), (10) and (11) and the results are shown in table 2.

The value for the heat of vaporization of methyl acetate at 25° calculated from the data given by Stull [18] is 8·0 kcal/mole. The value calculated from eqn. (6) is 7·4 kcal/mole. The difference is well within the expected error in eqn. (6) but the value from the equation is low indicating that the difference might be attributed to an increase in charge density in the smaller methyl acetate molecule not accounted for in the equation. This would increase the heat of vaporization.

In a similar way, the heat of vaporization of acetic acid at 25° calculated from vapour pressure data [18] is 9·8 kcal/mole. Eqn. (4) gives 12·6 kcal/mole. There are two effects operating here: (i) increase in the heat of vaporization due to increased charge density in the smaller acid molecule; the value for this effect can be estimated as equal to the same value found for methyl acetate—0·6 kcal/mole; (ii) hydrogen bonding in the vapour phase for acetic acid is about 80 % complete at 25° as can be calculated from the equilibrium constant for this hydrogen bonding [19]; the value for this effect can be set equal to 5·6 kcal/mole (7·0 × 0·8) and subtracted since hydrogen bonding in the vapour lowers the heat of vaporization. Applying these two corrections gives a heat of vaporization of 7·6 kcal/mole compared with the value 9·8 kcal/mole calculated from the data.

TABLE 2.—CALC. AND OBS. HEATS OF VAPORIZATION AND SUBLIMATION OF LONG-CHAIN FATTY ACIDS AND THEIR METHYL ESTERS, kcal/mole

number of carbon atoms in chain	methyl esters					fatty acids			
	this work (liquid) 25°	Davies and Kybett[9] (solid)	calc. from ref. (10) and (16) (liq., 25°)	calc. using eqn. (5) or (6) 25°	calc. using 1·5 %/10° correction and ref. (9) 25°	from Spizzichino[10] for C-8 or this work 25°	Davies and Malpass[7] (solid)	calc. using eqn. (2), (3) or (4) 25°	calc. using 3 %/10° correction and ref. (7) and (11) 25°
8			13·6	14·0 (*l*)		23·5 (*l*)		19·2 (*l*)	20·3 (*l*)
9				15·1 (*l*)		19·7 (*l*)		20·3 (*l*)	
10			16·5	16·2 (*l*)		28·4 (*s*)	27·7 (17-28°)	28·4 (*s*)	27·7 (*s*)
11				17·3 (*l*)		29·0 (*s*)		29·0 (*s*)	
12		29·2 (−11-0°)	19·3	18·4 (*l*)		31·7 (*s*)	30·7 (23-41°)	32·6 (*s*)	30·7 (*s*)
13	19·5			19·5 (*l*)				33·2 (*s*)	
14		33·1 (0-16°)	21·4	20·6 (*l*)			33·7 (39-52°)	36·8 (*s*)	35·8 (*s*)
15	21·0			21·7 (*l*)				37·4 (*s*)	
16		37·0 (18-28°)		37·0 (*s*)	37·0 (*s*)		36·7 (47-60°)	41·0 (*s*)	39·8 (*s*)
17								41·6 (*s*)	
18		40·9 (26-37°)		41·2 (*s*)	41·3 (*s*)		39·7 (58-67°)	45·2 (*s*)	44·2 (*s*)
19								45·8 (*s*)	
20		44·8 (38-45°)		45·4 (*s*)	46·0 (*s*)		42·7 (64-73°)	49·4 (*s*)	48·2 (*s*)

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the time for computing these results

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COMPUTER ESTIMATION OF THE ATMOSPHERIC GAS-PHASE REACTION RATE OF ORGANIC COMPOUNDS WITH HYDROXYL RADICALS AND OZONE

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ABSTRACT

The Atmospheric Oxidation Program (AOP) is a computer program that estimates the rate constant for the atmospheric, gas-phase reaction between photochemically produced hydroxyl radicals (OH) and organic chemicals. It also estimates the rate constant for the gas-phase reaction between ozone and olefinic/acetylenic compounds. AOP, which uses estimation methods developed by Atkinson and co-workers, estimates more accurate rate constants than the PCFAP (Fate of Atmospheric Pollutants) program that was part of the U.S. EPA's Graphical Exposure Modeling System (GEMS). Due to its superior predictive ability, the EPA is currently using AOP to evaluate the atmospheric fate of compounds defined under Sections 4, 5 and 6 of the Toxic Substances Control Act (TSCA).

Introduction

Organic chemicals emitted into the troposphere are degraded by several important transformation processes that include reaction with hydroxyl (OH) radicals or other photochemically-produced radicals, reaction with ozone or direct photolysis[1-6]. The dominant transformation process for most compounds that occur in the troposphere is the daylight reaction with OH radicals[3,4]. For some olefinic structures, reaction with ozone is the major process[7]. The rates at which organic compounds react with OH radicals or ozone are a direct measure of their atmospheric persistence, and hence, their rates of reaction are needed to develop exposure assessments and the ozone depletion potential for halogenated compounds.

Rate constants have been measured experimentally for only a small fraction of the organic chemicals of environmental concern. The rate constant for the gas-phase reaction with OH radicals has

been measured for less than 500 organic compounds[4]. Since experimental measurements can be difficult, time-consuming and expensive, the ability to estimate rate constants has become increasingly important[2] and estimation methodologies are of interest to regulatory agencies in preparing risk assessments of chemicals released to the atmosphere[3]. For example, when experimental data are missing at the screening level, the U.S. EPA must estimate OH radical rate constants for new and existing chemicals included under Sections 4, 5 and 6 the Toxic Substances Control Act (TSCA)[5,6]. This paper compares the accuracy of two computer programs used to estimate OH radical and ozone rate constants.

Estimation Methods and Programs

Two separate estimation methods and computer programs have been developed, at least in part, by research grants sponsored by the U.S. EPA. Both programs are based upon structure-activity relationships (SARs) and rely solely on the structure of organic chemicals. They estimate rate constants at 25°C. The first program is FAP (Fate of Atmospheric Pollutants) which was developed from the combined methods of Hendry, Kenley and Heicklen[8,9]. FAP is part of EPA's Graphical Exposure Modeling System (GEMS); the personal computer version of FAP is PCFAP. The second program is AOP (Atmospheric Oxidation Program) which is based on the methods of Atkinson and co-workers[1-3,10]. It is currently used by EPA's Exposure Assessment Branch to evaluate chemicals under Sections 4, 5 and 6 of TSCA[5] and by the German Environmental Protection Agency.

Both programs estimate an overall OH rate constant by summing individual OH reaction pathways that include hydrogen abstraction from aliphatic C-H groups, OH addition to olefins and acetylenes, and OH addition to aromatic rings. PCFAP and AOP calculate hydrogen abstraction by different procedures; PCFAP uses a procedure based on bond dissociation enthalpies whereas AOP uses a procedure based on substituent connections to CH_3, CH_2 or CH groups. AOP adds several pathways not included in PCFAP such as hydrogen abstraction from OH groups and OH radical interactions with nitrogen, phosphorus and sulfur. In addition, AOP can detect differences in aromatic rings while PCFAP can not; for example, PCFAP treats pyridine or triazine as benzene.

Although time-consuming, it is possible to "hand-calculate" a rate constant using the methods in AOP and PCFAP given a thorough knowledge of the methods. A much easier procedure is to enter the structure of a compound into a computer program as a SMILES (Simplified Molecular Input Line Entry System) notation, as in AOP and PCFAP. In both programs, a database of nearly 20,000 CAS Registry Numbers can be used to automatically enter SMILES notations. Once a SMILES in entered, AOP performs the calculation in less than one second.

Accuracy of Estimates for Reaction with OH Radicals. A list of 448 compounds with measured OH rate constants was located using Syracuse Research Corporation's Environmental Fate Data Base (EFDB)[11,12]. The bulk of this list was taken from a recent compilation by Atkinson[4]. The AOP and PCFAP programs were then used to estimate rate constants for all 448 compounds and the estimates were compared to the experimental values. For the AOP estimations, 90% are within a factor of two of

the experimental value and 95% are within a factor of three. For the PCFAP estimations, only 49% are within a factor of two of the experimental value and only 66% are within a factor of three. Since the range of experimental rate constants spans nearly six orders of magnitude, a statistical correlation was computed on a logarithmic basis. Comparing experimental to estimated value, AOP has a correlation coefficient (r^2), standard deviation and mean error of 0.96, 0.21 log units and 0.12 log units, respectively; PCFAP has a correlation coefficient, standard deviation and mean error of 0.51, 0.80 log units and 0.52 log units, respectively. PCFAP is particularly inaccurate when estimating compounds containing phosphorus, sulfur (such as thiols, sulfides and aromatic sulfurs), or nitrogen (such as nitriles, nitro functions, aromatic nitrogens, or amines). Figures 1 and 2 illustrate AOP and PCFAP's correlation with experimental values.

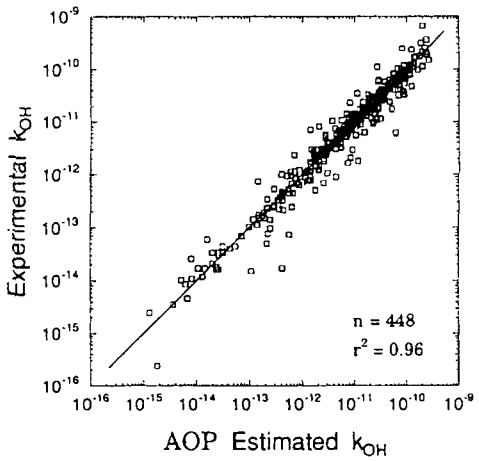

Figure 1. AOP estimates vs experimental OH rates (in cm^3/molecule-sec)

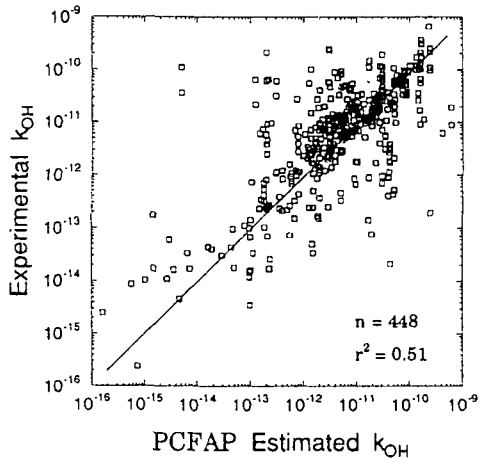

Figure 2. PCFAP estimates vs experimental OH rates (in cm^3/molecule-sec)

Since most of the 448 compounds in the experimental list were used to derive either the AOP or PCFAP methodologies, the accuracy suggested by the above statistics may not adequately describe the overall method accuracy. A more legitimate test of an estimation method is the ability to predict accurate values for an independent test set of chemicals that were not used in developing the method. From the 448 compounds, 77 compounds were located that were not used to derive either method. For the 77 compounds, AOP has a correlation coefficient (r^2), standard deviation and mean error of 0.89, 0.24 log units and 0.17 log units, respectively; PCFAP has a correlation coefficient, standard deviation and mean error of 0.44, 0.56 log units and 0.42 log units, respectively. Table 1 lists a representative portion of the 77 compounds.

Table 1. Comparison of experimental and estimated OH radical rate constants for a partial list of compounds used to test the accuracy of AOP and PCFAP (rate constants reported in units of 10^{-12} cm^3/molecule-sec)

	AOP	Experimental	PCFAP
2,2-Dimethylpentane	3.22	3.37	1.56
4-Methyloctane	9.95	9.72	5.70
n-Pentadecane	17.87	22.2	7.47
Isopropylcyclopropane	2.85	2.84	3.25
Cyclooctane	11.16	13.7	4.62
Pentane-1,5-dial	46.9	23.8	47.5
Cyclohexanone	12.55	6.39	2.92
2,5-Hexanedione	5.82	7.13	1.35
Hydroxyacetone	2.31	3.02	3.17
Methoxyacetone	4.87	6.77	10.62
1,1,1-Trifluoroacetone	0.109	0.0151	0.0978
1-Pentanol	7.77	10.8	4.89
Cyclopentanol	12.78	10.7	15.78
2-Methoxyethanol	11.18	12.5	13.72
2,2,2-Trichloroethanol	0.29	0.245	2.92
2,2,2-Trifluoroethanol	0.25	0.0955	0.0475
Ethyl n-butyl ether	18.5	18.1	19.2
Methyl tert-amyl ether	6.13	7.91	2.38
1,4-Dioxane	26.4	10.9	36.0
3-Methylfuran	106.6	93.5	5.1
Isobutyric acid	1.80	2.00	2.81
n-Propyl formate	2.89	2.4	1.32
Methyl trifluroacetate	0.216	0.05	1.48
n-Propyl propionate	3.07	4.0	1.68
n-Propyl butyrate	4.16	7.4	2.24
2-Butyne	29.29	27.4	1.81
Di-n-propyl sulfide	24.0	20.0	2.50
Dimethylnitramine	2.88	3.84	0.20
t-Butylbenzene	5.08	4.60	5.29
Acephenone	1.61	2.74	5.10
Benzyl alcohol	7.99	22.9	7.98
2,5-Dimethylphenol	115.4	80.0	30.2
2,6-Dimethylphenol	54.1	65.9	30.2
2-Nitrotoluene	0.81	0.70	30.1
1-Nitronaphthalene	2.7	5.4	34.0

Chlorofluorocarbons (CFCs) have raised an environmental concern due to possible destruction of the atmospheric ozone layer. Table 2 compares estimates from AOP and PCFAP to experimental values for various CFCs. With the exception of 1,1,1-trifluoroethane, AOP produces much better estimates.

Table 2. Comparison of experimental and estimated OH radical rate constants for a list of chlorofluorocarbons.
(rate constants reported in units of 10^{-12} cm^3/molecule-sec)

	AOP	Experimental	PCFAP
Chlorofluoromethane	0.0315	0.0441	0.0164
1,1-Difluoroethane	0.0323	0.0034	0.114
1,1,1-Trifluoroethane	0.0108	0.0017	0.0015
1,1,2-Trifluoroethane	0.0235	0.018	0.050
1-Chloro-1,1-difluroethane	0.0036	0.00358	0.099
1,1,1-Trichloroethane	0.013	0.0119	0.097
1,1,2-Trichloroethane	0.332	0.328	0.681
1,1,1,2-Tetrafluoroethane	0.0062	0.006	0.00057
1,1-Dichloro-1-fluoroethane	0.013	0.007	0.0976
1-Chloro-2,2,2-trifluoroethane	0.0239	0.0162	0.00361
1,2-Dichloro-2,2-difluoroethane	0.008	0.026	0.239
Pentafluoroethane	0.0013	0.0025	0.000164
1-Chloro-1,2,2,2-tetrafluoroethane	0.0052	0.0102	0.00104
1,1-Dichloro-2,2,2-trifluoroethane	0.020	0.0335	0.00659
1-Bromo-1-chloro-2,2,2-trifluoroethane	0.016	0.060	0.003

Accuracy of Estimates for Reaction with Ozone. AOP and PCFAP can estimate rate constants for the gas-phase reaction between ozone and olefinic or acetylenic compounds. A list of 79 olefins and acetylenes with measured ozone rate constants was located using Syracuse Research Corporation's Environmental Fate Data Base (EFDB)[11,12]. Many from this list were taken from a compilation by Atkinson and Carter[7]. The AOP and PCFAP programs were then used to estimate rate constants for all 79 compounds and the estimates were compared to the experimental values. Similar to the OH values, the range of experimental rate constants for ozone spans nearly six orders of magnitude, so the statistical correlation was computed on a logarithmic basis. Comparing experimental to estimated value, AOP has a correlation coefficient (r^2), standard deviation and mean error of 0.93, 0.42 log units and 0.27 log units, respectively; PCFAP has a correlation coefficient, standard deviation and mean error of 0.72, 0.88 log units and 0.71 log units, respectively. PCFAP is particularly inaccurate for substituted olefins where the substitutions are halogens or functional groups containing oxygen. Table 3 compares estimates from AOP and PCFAP to experimental values for various haloalkenes.

Table 3. Comparison of experimental and estimated ozone rate constants for haloalkenes.
(rate constants reported in units of 10^{-18} cm^3/molecule-sec)

	AOP	Experimental	PCFAP
Vinyl fluoride	0.70	0.70	1.90
1,1-Difluoroethene	0.28	0.19	1.90
trans-1,2-Difluoroethene	0.28	0.21	1.90
Trifluoroethene	0.112	0.14	1.90
Tetrafluoroethene	0.045	0.092	1.90
Vinyl chloride	0.25	0.24	1.90
Hexafluoropropene	0.0112	0.0077	13.0
cis-1,3-Dichloropropene	0.0113	0.015	13.0
2-(Chloromethyl)-3-chloro-1-propene	0.142	0.039	13.0

Conclusions

AOP is clearly superior to FAP as demonstrated by its ability to estimate more accurate values for both OH radicals and ozone rate constants. Leifer[5,6] has critically evaluated the available SAR methods for estimating OH radical rate constants and found the methods used in AOP (the Atkinson SARs) to be the most accurate of all methods and applicable to the widest number of structures. In addition, the Atkinson SARs were adopted by OECD (Organization for Economic Cooperation and Development) in 1988 to be used as guidance when performing gas-phase transformation tests[5]. An independent evaluation has found the Atkinson SAR method and AOP software to produce generally good estimates[13].

How to Obtain the Programs

PCFAP is available from General Sciences Corporation, 6100 Chevy Chase Drive, Laurel, MD 20707. The AOP program is available from the corresponding author at the address indicated above.

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IMPROVED METHOD FOR ESTIMATING BIOCONCENTRATION/BIOACCUMULATION FACTOR FROM OCTANOL/WATER PARTITION COEFFICIENT

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Abstract—A compound's bioconcentration factor (BCF) is the most commonly used indicator of its tendency to accumulate in aquatic organisms from the surrounding medium. Because it is expensive to measure, the BCF is generally estimated from the octanol/water partition coefficient (K_{ow}), but currently used regression equations were developed from small data sets that do not adequately represent the wide range of chemical substances now subject to review. To develop an improved method, we collected BCF data in a file that contained information on measured BCFs and other key experimental details for 694 chemicals. Log BCF was then regressed against log K_{ow} and chemicals with significant deviations from the line of best fit were analyzed by chemical structure. The resulting algorithm classifies a substance as either nonionic or ionic, the latter group including carboxylic acids, sulfonic acids and their salts, and quaternary N compounds. Log BCF for nonionics is estimated from log K_{ow} and a series of correction factors if applicable; different equations apply for log K_{ow} 1.0 to 7.0 and >7.0. For ionics, chemicals are categorized by log K_{ow} and a log BCF in the range 0.5 to 1.75 is assigned. Organometallics, nonionics with long alkyl chains, and aromatic azo compounds receive special treatment. The correlation coefficient (r^2 = 0.73) and mean error (0.48) for log BCF (n = 694) indicate that the new method is a significantly better fit to existing data than other methods.

Keywords—Bioconcentration factor Bioconcentration Octanol/water partition coefficient Estimation

INTRODUCTION

Organisms may accumulate chemical substances either directly from the surrounding environment or from their diet. Nondietary bioaccumulation in aquatic organisms is referred to as bioconcentration, and may be viewed as a process in which the substance is distributed between the organism and the environment in accordance with the substance's chemical properties, environmental conditions, and biological factors such as the organism's ability to metabolize the substance. The tendency of a waterborne substance to bioconcentrate in aquatic organisms is usually expressed as its bioconcentration factor (BCF), which is formally defined as the equilibrium ratio of the concentration of the substance in the exposed organism to the concentration of the dissolved substance in the surrounding environment [1]. The BCF is an important parameter in environmental assessment. Bioconcentration emerged as an ecological concern more than 40 years ago when studies demonstrated that accumulation of pesticide residues in fish had led to reproductive failure in piscivorous birds [2]. Humans are also consumers of fish and shellfish, and exposure assessments now routinely consider fish ingestion as potential route of human exposure to chemicals in the environment [3,4].

Standardized protocols for determining BCFs exist for several aquatic species, for example, the U.S. Environmental Protection Agency's (U.S. EPA's) recently updated fish and oyster BCF test guidelines [5,6] and the guidelines of the Organization for Economic Cooperation and Development and American Society for Testing and Materials, as summarized by the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) [7]. In general, however, experimental determination of the BCF is expensive and demanding if performed correctly. Because of this, measuring the BCFs of the many thousands of chemical substances that are of potential regulatory interest simply is not possible. This is obviously true for new chemicals such as those reviewed under the U.S. Toxic Substances Control Act, but it is equally applicable in the context of numerous chemical ranking and scoring exercises that are currently being undertaken worldwide with the aim of identifying chemicals that may be persistent, bioaccumulative, and toxic [8,9]. As with so many other parameters needed for screening-level assessment, estimation methods have and will continue to play a critical role in supplying the missing data.

The BCF is usually estimated from regression equations of the general form

$$\log \text{BCF} = a \log K_{ow} + b \quad (1)$$

where a and b are empirically determined constants and K_{ow} is the n-octanol/water partition coefficient. The assumption is that bioconcentration is a thermodynamically driven partitioning process between water and the lipid phase of the exposed organism, and therefore can be modeled using n-octanol as a surrogate for biological lipids. Numerous versions of Equation 1 have been published, but the broadly applicable (i.e., not class-specific) equations generally have slopes close to 1 and negative intercepts [10–13]. For example, the U.S. EPA Office of Pollution Prevention and Toxics (OPPT) currently uses the equation of Veith and Kosian [14] in which a = 0.79 and b = −0.40. Such equations give a fair approximation of the BCF for nonionic, nonmetabolized substances with log K_{ow} in the range of 1 to 6, but the relationship breaks down with more

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hydrophobic substances as well as substances that are metabolized to an appreciable extent by the exposed organism [1,15]. Another consideration is that the equation of Veith and Kosian was developed from only 122 measured BCF values for 60 organic compounds [14]. Other published correlations with log K_{ow} are similarly based on small training sets relative to currently available data.

Connell and Hawker [16] used a polynomial to describe the relationship of log BCF and log K_{ow}, and Bintein et al. [17] proposed a bilinear model for BCF prediction of the form described by Kubinyi [18]. This model provides a much better fit to measured BCFs for substances with log K_{ow} values >6 than do simple linear regressions against log K_{ow}. However, the Bintein training set (154 compounds) was only moderately larger than that of Veith and Kosian, and Bintein et al. [17] did not attempt to correlate estimation error with specific aspects of molecular structure. Our objectives, therefore, were threefold: first, assemble a large and relatively comprehensive database of evaluated fish BCF data and associated log K_{ow} values for use in modeling; second, develop an improved screening-level estimation method based on log K_{ow} and other factors, especially molecular structure and functional groups that dissociate; and third, encode the improved method in a user-friendly computer program.

METHODS

BCF data

Measured BCF values were obtained primarily from the U.S. EPA's online AQUIRE database [19]; a large database of BCF values collected by the Japanese Chemicals Inspection and Testing Institute (CITI) [20], which is available only in hard copy format; the National Library of Medicine's Hazardous Substances Data Bank (HSDB) [21]; and sources referenced in the Environmental Fate Data Base (EFDB) [22,23], which is maintained by Syracuse Research Corporation (SRC) (North Syracuse, NY, USA). Most data were retrieved from AQUIRE and CITI (277 and 479 compounds, respectively). Only fish BCF data were collected for this study. We retrieved AQUIRE data first followed by CITI data, and then used the HSDB and the DATALOG file of the EFDB to identify any additional BCF values and chemicals not in the first two sources, so that a relatively comprehensive record could be developed before selection of a recommended value for modeling.

Log K_{ow} data

Measured values of K_{ow} were retrieved from the MEDCHEM "star list" [24], Sangster's LOGKOW DATABANK ("recommended" values only) [25], or sources referenced in the EFDB [22,23]. If a measured value was unavailable (generally true for the most hydrophobic chemicals), log K_{ow} was estimated using SRC's program LOGKOW© (the version for Microsoft Windows™ is KOWWIN©), which uses an atom/fragment contribution methodology [26]. Measured log K_{ow} values were available for most of the chemicals in the BCF database.

The BCF database contained 30 sodium salts and one potassium salt, and measured log K_{ow} values were not available for any of these compounds. Our solution was to use KOWWIN to estimate log K_{ow} for the nonsalt forms of these compounds and use these values in developing the new method for estimating the BCF. For example, the log K_{ow} for sodium naphthalenesulfonate was estimated by entering the Simplified Molecular Identification and Line Entry System (SMILES) notation for naphthalenesulfonic acid (KOWWIN can also estimate log K_{ow} for the salt form). In general, the difference between log K_{ow} values for salt and nonsalt forms is substantial, as illustrated by pentachlorophenol and pentachlorophenate for which the values are 5.12 and 2.05, respectively. On the other hand, measured log BCF values for the two forms are nearly identical at 2.89 and 2.81, respectively, and these values are more consistent with the undissociated form being the chief form that is bioconcentrated.

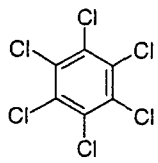
pK_a data

Experimentally determined dissociation constants (pK_a) for ionizing compounds were obtained from a variety of sources, including SRC's PHYSPROP© database [27], the extensive compilations of Perrin [28,29] and Serjeant and Dempsey [30], reference books such as the *Handbook of Organic Chemistry* [31] and the *Handbook of Chemistry and Physics* [32], and other sources cited in the EFDB [22,23]. If a measured value was unavailable, pK_a was estimated using either pKalc 3.1 software from CompuDrug [33] or the chemical property and reactivity expert system SPARC [34].

BCF database

The initial database contained experimental fish BCF data for 727 compounds: this differs from 277 AQUIRE chemicals + 479 CITI chemicals + EFDB and HSDB chemicals because of overlap among the data bases. The BCF file was constructed using ISIS™/Base software [35] to allow convenient searching by molecular substructure. Figure 1 shows a typical record, in this case for hexachlorobenzene (Chemical Abstracts Service [CAS] 118-74-1). Each record has fields for the CAS registry number, chemical name, molecular structure diagram, log K_{ow}, pK_a if applicable, measured log BCF values plus partial reference citations and key experimental details if available, a single recommended log BCF selected from the listed values, and a comment briefly explaining the rationale for selecting the recommended value. Multiple log BCF values were available for most of the compounds in the database. For a few chemicals, such as the example in Figure 1, not all existing data are listed in the record for that chemical because we limited records to a maximum of 10 individual measured values. Experimental details captured in the database included fish species, exposure concentration of test compound, percent lipid of the test organism, test method (equilibrium exposure vs kinetic method), test duration if equilibrium method, and tissue analyzed for test compound (whole body, muscle filet, or edible tissue).

Recommended values for use in regression analysis were selected from the listed data for each compound according to a set of general guidelines. Data obtained by the kinetic method were preferred to data from the equilibrium method, especially for compounds with high log K_{ow} values, which are less likely to have reached equilibrium in standard tests. For equilibrium-method studies a BCF value in the middle of the range of values with the longest exposure times was selected, especially for compounds with high log K_{ow} values (same reason as noted above). Low exposure concentrations of test compound were favored in order to minimize the potential for toxic effects and maximize the likelihood that the total concentration of the substance in water was equivalent to the bioavailable fraction. Data obtained under flow-through conditions were selected whenever possible. Data were rejected if significant contam-

CAS # 000118-74-1	HEXACHLOROBENZENE
Recommended Log BCF: 4.27	
Log Kow 5.73 / pKa	
Comment: Recommended log BCF was for fathead minnow from Veith - was in the middle of the range of other fathead minnow results.	

Log BCF	Species	Conc ug/L	Lipid%	Method*	Tiss**	Reference
4.37	Rainbow trout			(4)	M	Veith,GD & P. Kosian 1983, DTLG
3.78 - 4.48	Carp	0.05 - 0.50		E(56)	W	CHEMICALS INSPECTION AND TESTING INSTITU (1992)
4.34	Fathead minnow	0.30 - 4.80		(32)		Carlson,A.R. and P.A.Kosian 1987, AQ12124
4.27	Fathead minnow	2.60		(32)		Veith,G.D., D.L.Defoe, and 1979, AQ616
4.16	Guppy			(19)	W	Veith, GD & P. Kosian 1983, DTLG
4.37	Fathead minnow			(30)	W	Veith, GD & P. Kosian 1983, DTLG
4.19 - 4.33	Fathead minnow	0.30 - 3.80		(28)		Nebeker,A.V., W.L.Griffis, 1989, AQ760
3.23 - 3.79	Rainbow trout	3.10 - 4.50		(4-30)		Murty,A.S. and P.D.Hansen 1983, AQ12479
3.73 - 4.59	Fathead minnow	0.14 - 0.19		(2-28)		Kosian,P., A.Lemke, 1981, AQ81
4.97 - 4.98	Fathead minnow	3.50 - 5.00		(28)		Schuytema,G.S., D.F.Krawczyk, 1990, AQ3005

Footnotes: *Method - E, equilbrium exposure (days); K, kinetic method **Tissue - W, whole body; M, muscle fillet; E, edible tissue

Fig. 1. Bioconcentration factor database record for hexachlorobenzene, CAS 118-74-1.

ination of the exposure medium by food, excreta, or other adsorbents was suspected, because this may reduce the bioavailability of the test compound. Warm-water fish were preferred to cold-water fish because more data were available for warm-water species. Fish species were preferred in the order fathead minnow > goldfish > sunfish > carp > marine species (this list is not all inclusive). Fathead minnow data were generally selected over data from other species because such data were available for a large number of chemicals, and because they have been used to develop log K_{ow}-based BCF estimation methods in previous studies [10,11]. Because dietary accumulation may have contributed to total uptake in some studies, especially for high-log K_{ow} chemicals, for the present study it may be more appropriate to describe the BCF as a bioconcentration/bioaccumulation factor.

Using hexachlorobenzene (Fig. 1) as an example, the comment field indicates that all 10 measured BCF values listed in the record were for freshwater fish and were retrieved from either the AQUIRE [19] or the CITI database [20]. From these values we selected a log BCF value of 4.27 from a fathead minnow study [10], because fathead minnow data were preferred over other data and this value was in the middle of the range of all listed data for this species. Note that because AQUIRE does not indicate directly whether an equilibrium or kinetic method was used, the BCF record in Figure 1 states only the exposure time, in this case 32 days. The AQUIRE database also does not give information on lipid content. The CITI database does state lipid content in most cases, although not in the present example. Recommended log BCF values were not corrected for lipid content.

Before development of the new BCF estimation method we eliminated 33 compounds from the 727-compound database. Compounds were generally excluded for one of two reasons: no measured log K_{ow} value was available and estimated values were deemed not reliable for the subject compound; or the BCF value initially recommended for use in modeling was later judged to be of questionable validity, for example, if the record for that chemical contained only a single measurement for an "unusual" species of fish or if the test period was considered too short. The resulting file contained 694 discrete compounds and was used for regression modeling.

Statistical analysis

All regressions and statistical operations were performed using CoStat Statistical Software [36] on an IBM-compatible 100 MHz Pentium MS-DOS personal computer.

RESULTS

Nonionic compounds

Figure 2 shows the relationship between recommended log BCF and log K_{ow} for the complete database of 694 compounds. Also depicted are Veith and Kosian's linear relationship between log K_{ow} and log BCF [14] (solid line) and the bilinear relationship developed by Bintein et al. [17] (dashed line). The equations of Bintein and Veith and Kosian yield similar results for compounds with log $K_{ow} < 6$; however, as expected, the bilinear equation provides a much better fit to the data for log $K_{ow} > 7$. Although low BCF values suggest that bioaccumulation is not of any practical concern, from a modeling perspective it is noteworthy that both equations also underestimate log BCF somewhat at log $K_{ow} < 0.5$.

We used these observations as a starting point in developing a new method for estimating log BCF. The 694 compounds were first divided into data sets containing nonionic and ionic compounds. For the nonionic compounds the relationship between log BCF and log K_{ow} appeared to be linear in the log K_{ow} range of 1 to 7, so we retained the linear form expressed

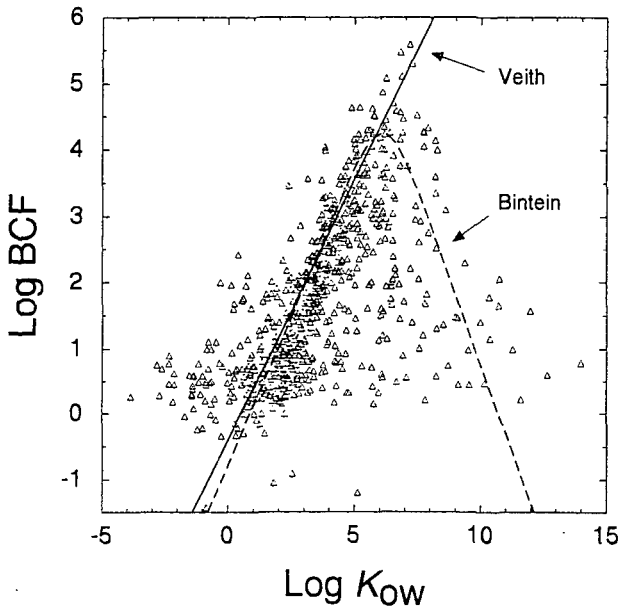


Fig. 2. Measured BCF versus log K_{ow} for the 694-compound BCF database. The solid line represents the linear equation of Veith and Kosian [14]; the dashed line represents the bilinear equation of Bintein et al. [17].

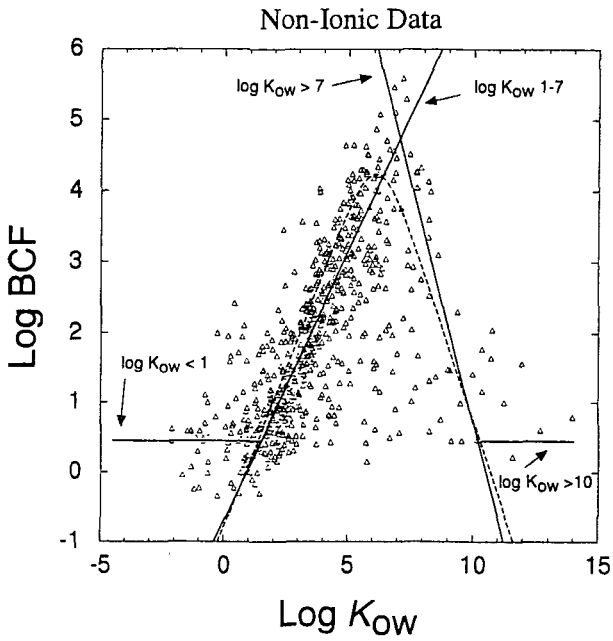


Fig. 3. Measured log BCF versus log K_{ow} for 610 nonionic compounds. The solid lines represent the equations used by the new BCF estimation method for the indicated log K_{ow} ranges; the dashed line represents the bilinear equation of Bintein et al. [17].

in Equation 1 for that log K_{ow} range. The resulting equation is

$$\log BCF = 0.86 \log K_{ow} - 0.39 \quad (2)$$

Analysis of the residuals from Equation 2 for the log K_{ow} range 1 to 7 revealed that compounds sharing certain structural features also tended to have residuals that were relatively consistent in sign and magnitude. On this basis we identified several compound classes that seemed amenable to derivation of correction factors. We then added indicator variables for these classes to the variable log K_{ow} and reran the regression as a multiple linear regression. The resulting equation is

$$\log BCF = 0.77 \log K_{ow} - 0.70 + \sum F_i \quad (3)$$

where ΣF_i is the summation of all correction factors applicable to a given compound. The correction factors and rules for their application are given in the Appendix. Each factor either applies to a compound or it does not; that is, each factor is counted only once no matter how many times the functional group appears in the molecule. However, more than one correction factor may apply to a given compound and this was indeed the case for several compounds in the database.

Linear equations were also derived for log $K_{ow} > 7$ and <1. For log $K_{ow} < 1$, because most measured log BCF values are in the range of 0 to 1.0, we decided to use a constant log BCF value of 0.5 (i.e., horizontal line on the plot of log BCF vs log K_{ow} at BCF = 0.5) to describe the relationship between log BCF and log K_{ow}. For log $K_{ow} > 7$, the linear equation of best fit for nonionic compounds is

$$\log BCF = -1.37 \log K_{ow} + 14.4 + \sum F_i \quad (4)$$

where the correction factors and application rules summarized in the Appendix also apply. Estimates of log BCF from Equation 4 must be truncated at 0.5 (i.e., the equation used is log BCF = 0.5) because negative values are otherwise derived when log $K_{ow} > 10.5$. Figure 3 shows the relationship between log BCF and log K_{ow} for 610 nonionic compounds, including solid lines for the four linear equations discussed above and a dashed line for the bilinear equation of Bintein et al. [17]. Statistics for estimation of log BCF of 610 nonionic compounds by the new method (four equations), the equation of Veith and Kosian [14], and the bilinear equation [17] are given in Table 1. Although the Bintein equation uses a single mathematical expression to describe the relationship between log BCF and log K_{ow}, it can be approximated by two linear equations that are nearly the same as the linear equations for log $K_{ow} > 7$ and $1 <$ log $K_{ow} < 7$ in the new method. The equation of Veith and Kosian [14] is also very similar to the present method and that of Bintein et al., in the log K_{ow} range of 1 to 7. From Table 1 it is evident that the new method provides a considerably better fit to the data set of recommended BCF

Table 1. Statistics for estimated versus measured log BCF

Statistic[a]	New method	Veith and Kosian [14]	Bintein et al. [17]
Nonionic compounds			
n	610	610	610
r^2	0.73	0.31	0.52
SD	0.67	1.56	1.09
ME	0.48	1.04	0.83
Ionic compounds			
n	84	84	84
r^2	0.62	0.19	0.21
SD	0.41	2.03	1.90
ME	0.31	1.69	1.60
All compounds			
n	694	694	694
r^2	0.74	0.32	0.58
SD	0.65	1.62	1.25
ME	0.47	1.12	0.94

[a] r^2 = correlation coefficient; SD = standard deviation; ME = mean error.

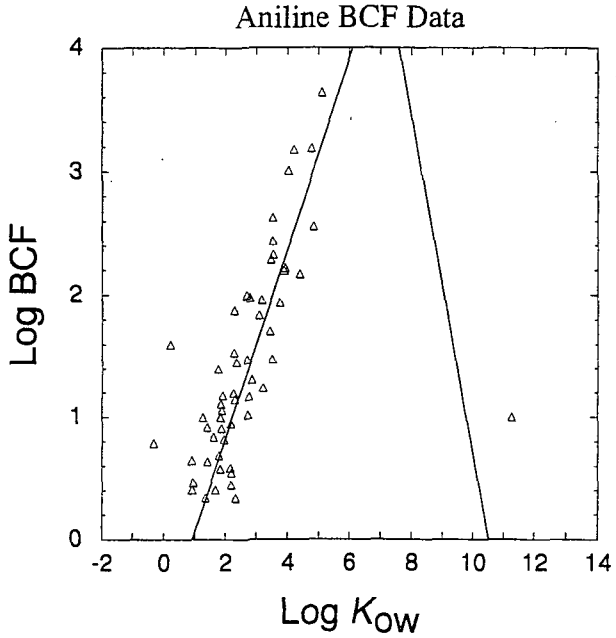


Fig. 4. Measured log BCF versus log K_{ow} for 56 anilines. The solid lines represent the two linear equations used by the new method for nonionics in the log K_{ow} ranges of 1 to 7 and >7.

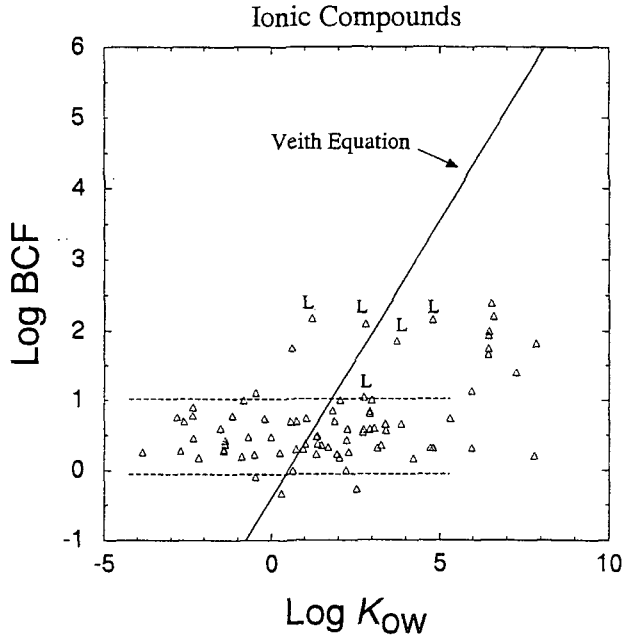


Fig. 5. Measured log BCF versus log K_{ow} for 84 ionic compounds. The solid line represents the equation of Veith and Kosian [14]. Points marked **L** represent compounds with long (≥11 carbons) alkyl chains; the dashed lines show that most compounds have log BCF values between 0 and 1.

values than the other methods, as shown by the higher correlation coefficient (higher r^2 value), but more importantly, a much lower standard deviation (SD) and mean error (ME).

Ionic compounds

The database of 694 compounds with recommended BCF values contained 84 compounds defined as ionic, including 41 carboxylic acids, 37 sulfonic acids and salts, and 7 quaternary ammonium compounds (i.e., compounds with nitrogen of +5 valence) (one compound is both a carboxylic and a sulfonic acid). Initially the 68 phenols and 56 anilines among the 694 compounds were assigned to the ionics subset, in part because pK_a data were available for most of these compounds and we were interested in determining whether log BCF for ionic compounds could be better fitted by including pK_a as an independent variable. However, subsequent analysis showed that the relationship of log BCF and log K_{ow} for these compounds is reasonably well described by the new method's equations for nonionics (for example, see Fig. 4), so they were assigned to this group.

Figure 5 shows the relationship between log BCF and log K_{ow} for the ionic compounds as defined here. It is obvious that the equation of Veith and Kosian [14], shown as a solid line in the figure, does not fit the data well (of course it was never intended to apply to ionic compounds). Because most of the recommended log BCF values for ionics with log $K_{ow} < 5$ were between 0 and 1, we decided to use the same basic approach as described above for nonionics with log $K_{ow} < 1$; that is, with one exception, a constant log BCF value of 0.5 is assigned to any ionic compound with log $K_{ow} < 5$. An exception is made for compounds with long alkyl chains, here defined as ≥11 carbons. The five ionics in the database that meet this criterion are denoted by an L in Figure 5 and although log K_{ow} for these compounds varies from 1.2 to 4.78, with one exception their log BCF values are all close to 2. Therefore, a lower limit of 1.85 (mean of recommended log BCF for the five compounds) is applied to estimates of log BCF for any such compounds.

For ionic compounds with log $K_{ow} > 5$, few recommended log BCF values were available, so instead of deriving a mathematical solution we developed a series of guidelines for assigning log BCF. The guidelines assume that log BCF increases up to log $K_{ow} = 7$ but then declines, as is the case for nonionics (Fig. 3), with log BCF being truncated at 0.5 for log $K_{ow} >$ 9. Specifically, for log $K_{ow} > 5$ the new method assigns log BCF values as follows: for log $K_{ow} < 5$, log BCF = 0.5; for $5 <$ log $K_{ow} < 6$, log BCF = 0.75; for $6 <$ log $K_{ow} < 7$, log BCF = 1.75; for $7 <$ log $K_{ow} < 9$, log BCF = 1.0; and for log $K_{ow} > 9$, log BCF = 0.5.

Statistics for estimation of log BCF of 84 ionic compounds by the new method, the equation of Veith and Kosian [14], and the bilinear equation of Bintein et al. [17] are given in Table 1. Although the observed correlation coefficient for the new method is considerably better than the corresponding statistics for the other two methods, the r^2 value (0.62) is still fairly low. However, SD and ME are better descriptors of method accuracy and indicate that the new method is actually relatively accurate for ionic compounds in the training set. We did examine both pK_a and molecular weight as independent variables in regressions of log BCF versus log K_{ow} for the ionics, but neither variable significantly improved the correlation.

Aromatic azo compounds

The BCF database contained eight ionics and seven nonionics classified as aromatic azo compounds. Log BCF did not appear to correlate with log K_{ow} for these compounds. Although log K_{ow} values ranged from −0.02 to 9.55, log BCFs were all in the range of 0.48 to 1.82. Therefore, the new method assigns a value of 1.0 (derived as the mean of the 15 recommended values in the training set) as the log BCF of nonionic aromatic azo compounds. Aromatic azo compounds that

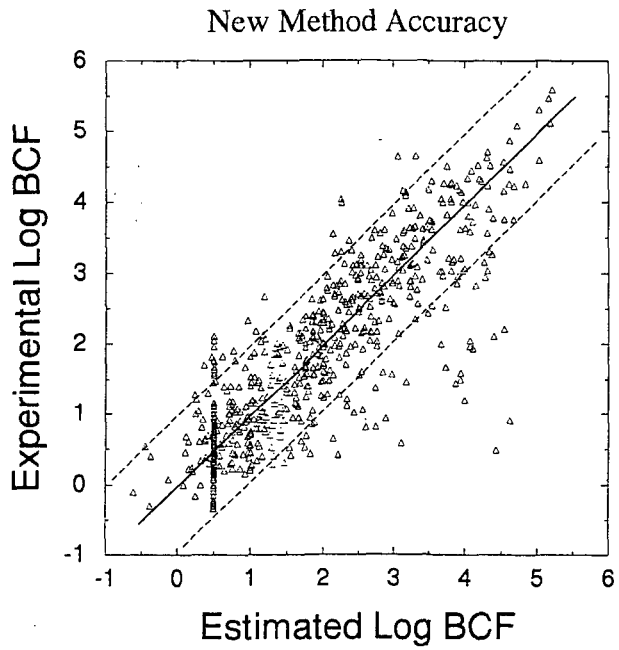


Fig. 6. Measured log BCF versus estimated log BCF for the 694-compound BCF database. The solid line represents the line of perfect fit; the dashed lines represent deviations from perfect fit of plus one and minus one log unit.

are ionic by definition are already handled by the ionic compound methodology just described, because most are dyes that are sulfonic acids or salts.

Tin and mercury compounds

Tin and mercury compounds also required special treatment. In general, log BCF appeared to be underpredicted by Equation 2, so a correction factor was developed for these compounds as indicated in the Appendix. However, even with the application of the correction factor, log BCF was significantly underpredicted at low values of log K_{ow} (<1). Because all 12 tin and mercury compounds had recommended log BCF values of approximately 2 or greater, we decided to truncate log BCF predictions at 2.0; that is, the new method assigns a minimum log BCF value of 2.0 regardless of log K_{ow} to any tin or mercury compound.

Accuracy for the combined data set

Table 1 summarizes statistics for the combined data set of 694 compounds with recommended log BCF values, and Figure 6 shows the relationship between recommended (experimental) log BCF and log BCF estimated by the new method. The solid line in Figure 6 indicates where all of the points would lie if experimental log BCF were perfectly predicted by the estimation methodology, and the dashed lines represent deviations from perfect fit of plus one and minus one log unit. For the combined data set of 694 compounds, 31, 55, 73, 84, and 90% of the estimates were within ±0.2, 0.4, 0.6, 0.8, and 1.0 log unit of the measured value, respectively. From Table 1 it is evident that the new method is approximately two and one-half times more accurate than the equation of Veith and Kosian [14] and twice as accurate as the equation of Bintein et al. [17] for compounds in the training set.

DISCUSSION

The results show that log BCF can be estimated to within one half log unit for the compounds in the training set, on average, using the methodology reported here. This level of accuracy is quite good considering the many sources of error that may impact this type of model. Nevertheless, estimates of log BCF should always be used with caution, especially when the estimated value is close to a level that triggers some regulatory or other action. The new method has been encoded in a computer program. BCFWIN©, and the methodology is summarized as follows:

Nonionic compounds

log K_{ow} < 1	log BCF = 0.50
log K_{ow} 1 to 7	log BCF = 0.77 log K_{ow} − 0.70 + $\sum F_i$
log K_{ow} > 7	log BCF = −1.37 log K_{ow} + 14.4 + $\sum F_i$
log K_{ow} > 10.5	log BCF = 0.50
Aromatic azo compounds	special treatment

Ionic compounds (carboxylic acids, sulfonic acids and salts, compunds with N of +5 valence)

log K_{ow} < 5	log BCF = 0.50
log K_{ow} 5 to 6	log BCF = 0.75
log K_{ow} 6 to 7	log BCF = 1.75
log K_{ow} 7 to 9	log BCF = 1.00
log K_{ow} > 9	log BCF = 0.50
Compounds with ≥C_{11} alkyl	special treatment

Tin and mercury compounds

Special treatment

The log K_{ow} value itself introduces some variability and this source of error needs to be more explicitly recognized [37]. Errors associated with log K_{ow} are especially likely for large molecules, charged or ionizable compounds, and surface-active agents. However, BCF measurement is probably a much larger source of error. Uncertainty associated with a given BCF measurement may arise from impurity of the test compound; the exposure concentration, which should be low enough to avoid any toxic effects and should remain constant during the experiment; the duration of the experiment, which should be sufficient for the test substance to achieve a steady-state concentration in the test organism; and determination of the concentration of test compound in water, which should reflect only the bioavailable concentration (dissolved fraction). Our methodology for selecting recommended log BCF values addressed these issues by emphasizing kinetic method studies and studies that minimized exposure concentration but maximized test duration. For all of these potential sources of uncertainty, experimental difficulties are more likely with hydrophobic chemicals, which are the very substances most likely to be of concern because of their tendency to bioaccumulate in aquatic organisms.

Bioavailability is a particularly thorny issue. The negative slope of measured log BCF versus log K_{ow} above log K_{ow} values of 6 to 7 has been attributed to a variety of factors, but Gobas et al. [38] suggested that much of this effect was likely due

to the total amount of solute in the water phase substantially exceeding the available (truly dissolved) fraction. Thus, if the measured BCF is calculated as the equilibrium ratio of solute in organism divided by total solute in water, the true magnitude of the BCF is underpredicted because the number in the denominator also includes sorbed (unavailable) solute. Geyer et al. [39] described an indirect method for estimating this "true" bioconcentration potential of superlipophilic chemicals, and showed that it does not actually decline as log K_{ow} increases beyond 6 to 7.

However, "true" bioconcentration potential as defined above may not reflect actual bioaccumulation under environmental conditions. Dietary uptake is likely to be more important than bioconcentration for very hydrophobic chemicals [40]. For example, a recent study by Fisk et al. [41] clearly showed that for rainbow trout exposed solely through their diet to 23 hydrophobic organochlorine compounds, bioaccumulation did decline at log K_{ow} values >7, consistent with simple relationships between log BCF and log K_{ow}. Moreover, recent studies [42,43] of toxaphene and PCB congeners in fish also found trends of decreasing bioaccumulation with increasing hydrophobicity, for the most hydrophobic congeners. Because both were monitoring studies of fish from sites contaminated for many years, the data presumably reflect the results of actual, long-term environmental exposures. This suggests that estimated BCFs for superhydrophobic chemicals derived from the new method may serve as acceptable surrogates for "true" bioaccumulation potential. Even so, we suggest that given all of the uncertainties, both measured and estimated BCFs for chemicals with log K_{ow} values above 6 to 7 be used only with caution and that the uncertainties always be acknowledged.

The selection of test species is also a source of error in estimation of BCFs. The process we used to collect fish BCF data and select recommended values for modeling emphasized fathead minnow data for practical reasons, but the database of recommended log BCF values intentionally included data for a wide variety of fish species. Only in this way was it possible to take advantage of the much larger universe of data and test compounds currently available, relative to the situation when the first log BCF/log K_{ow} correlations were published [2]. Despite this, the new method's equation for nonionics with log K_{ow} in the range of 1 to 7 is almost the same as an equation of Veith et al. [10] that was derived from just 55 log BCF values, all for fathead minnows

$$\log \text{BCF} = 0.85 \log K_{ow} - 0.70 \quad (5)$$

This suggests that despite differences between fish species with respect to metabolism of test compounds, lipid content, blood flow, and so on, for present purposes, combining data from different species is acceptable. Devillers et al. [44] similarly found that several previously published models relating log BCF to log K_{ow} that were based on a variety of fish species, including the bilinear model [17], yielded essentially equivalent results.

As further support for this approach, it is also interesting that the range of measured BCF values for hexachlorobenzene (Fig. 1) is as great for fathead minnow data alone as for all species combined. The same phenomenon was observed for many other compounds and it suggests that little would be gained in terms of accuracy by focusing on a single test species to the exclusion of others, even if the single-species data did cover a correspondingly large universe of chemical structures. In this context differences between species of the order of magnitude discussed by Barron [1] for hexachlorobenzene bioconcentration in rainbow trout versus fathead minnow (BCF values of 5,500 and 16,200, respectively) seem not so large, and indeed these two measurements differ by only 0.47 log unit (factor of three, approximately). Tsuda et al. [45] observed species differences of similar magnitude in a study of bioconcentration of 15 organophosphate pesticides in guppies, killifish, goldfish, and white cloud mountain fish.

Much attention has been paid in recent bioconcentration research to the effects of interspecies variation in lipid content and biotransformation capabilities. We did not correct BCF values for fish lipid content primarily because these data were not available in most publications. Moreover, a cursory inspection of BCF data in our database did not reveal any strong patterns with respect to log BCF values in warm-water fish versus cold-water fish, which might be expected to have a higher fat content on average. Normalizing to lipid content can eliminate some interspecies variation in BCF values, but it has been suggested that lipid content may not be as important a determinant of bioconcentration potential as previously thought [1].

In aquatic animals, biotransformation of a chemical substance assimilated from an animal's environment has the effect of increasing the substance's net elimination rate and thus decreasing the equilibrium level of the substance in the organism. Fish have the same complement of enzymes as do mammals [1], including those catalyzing phase I (oxidative, reductive, and hydrolytic) as well as phase II (conjugative) reactions, although activity is often lower [46]. In theory this should ordinarily lead to observed BCF values that are lower than predicted from linear regressions based on log K_{ow} alone and thus appear as outliers below the line of best fit for log BCF versus log K_{ow}. A significant body of evidence now supports the role of metabolism in producing BCF values that are lower than expected. Examples include benzo[*a*]pyrene [47], chlorinated anilines [48], and certain organophosphates [49].

Our original intention was to incorporate metabolism into a general estimation model for log BCF by defining correction factors for chemical classes for which it is known to have a significant influence. In addition, we hoped to use published studies of in vivo and in vitro fish metabolism of various insecticides to identify, a priori, chemical classes for which correction factors might be developed. Certain correction factors in the Appendix, for example those for cyclopropyl esters (permethrins) and compounds with tert-butyl groups ortho to a phenolic OH, can indeed be rationalized on the basis of either known biotransformation reactions (permethrins are oxidized by P450 in rainbow trout) [46] or likely reactivity (compounds with tert-butyl groups ortho to phenolic OH are antioxidants). But as a rule the basis for identifying chemical classes for correction factors was empirical and not based on first principles. For example, the data for anilines in Figure 4 show that simple regressions of log BCF versus log K_{ow} work quite well for these chemicals. These results can probably be attributed to the fact that for many metabolizable compounds, metabolism results in deviations from the expected BCF values of less than a factor of 10, which may be difficult to discern given the overall accuracy of the new method. Examples of much larger deviations due to metabolism exist [50], and could form the basis of additional correction factors given sufficient data.

We expended considerable effort to determine whether pK_a

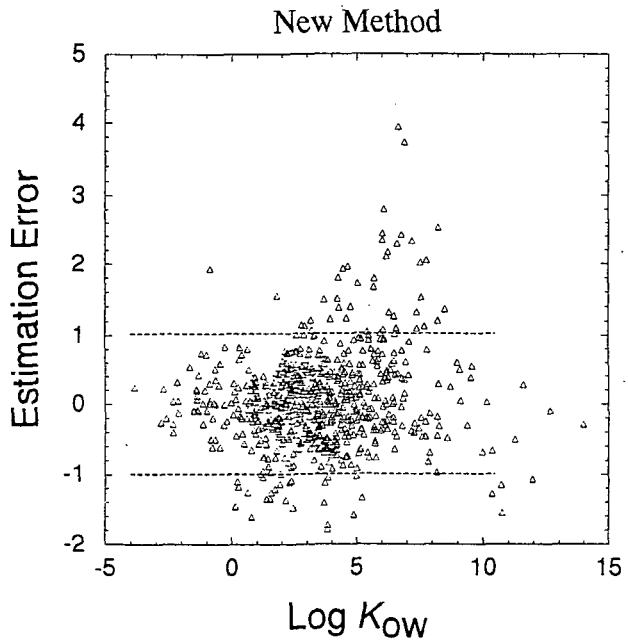


Fig. 7. Estimation error versus log K_{ow} for the 694-compound BCF database, using the new estimation method.

could improve the goodness of fit, not only for the entire database but also in regressions for specific classes such as phenols, but in no case did inclusion of this variable significantly improve results. In part this may be attributable to the methodology, which combined data for many different fish species and from many different sources. Correlation of log BCF not with log K_{ow} but rather with the log of the distribution coefficient D, calculated from a compound's pK_a and the medium pH using the Henderson–Hasselbalch equation [51], possibly could yield an improved correlation for ionizable compounds. However, as with % lipid and other potentially useful data, pH is often not reported and was not available, for example, in the AQUIRE and CITI databases.

The 694-compound data set used to develop the new BCF estimation method may still contain some outlier data that could either be eliminated or modeled with additional correction factors, and this should be a focus of further work. If compounds with estimation errors exceeding one log unit are set aside, the overall regression statistics become $n = 621$, $r = 0.95$ ($r^2 = 0.89$), SD = 0.41, ME = 0.34; which are comparable to statistics for several other models summarized by Devillers et al. [44] despite the much larger and more diverse data set used here. Figure 7 shows that nearly all positive outliers (i.e., compounds for which estimated log BCF substantially exceeds the recommended measured value) are in the log K_{ow} range of 4 to 9. Overprediction of BCF for these compounds may reflect biotransformation, but because of the many sources of potential error associated with very hydrophobic compounds, as noted above, it seems unnecessary to invoke metabolism as the explanation.

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APPENDIX

Correction factors for BCF estimation

Correction factor	Value
Compounds with an aromatic *s*-triazine ring (three compounds)	−0.32
Compounds containing an aromatic alcohol (e.g., phenol) with two or more halogens attached to aromatic ring (17 compounds)	−0.40
Compounds containing an aromatic ring with a *tert*-butyl group in a position ortho to an –OH group (e.g., *tert-butyl ortho*-phenol) (six compounds)	−0.45
Compounds containing an aromatic ring and an aliphatic alcohol in the form of –CH–OH (e.g., benzyl alcohol) (four compounds)	−0.65
Phosphate ester, O=P(O–R)(O–R)(O–R), where R is carbon (one R can be H) (18 compounds)	−0.78
Ketone with one or more aromatic connections (18 compounds)	−0.84
Nonionic compounds with an alkyl chain containing 8 or more $-CH_2-$ groups (13 compounds)	−1.00 ($\log K_{ow}$ of 4–6) −1.50 ($\log K_{ow}$ of 6–10)
Compounds containing a cyclopropyl ester of the form cyclopropyl–C(=O)–O– (e.g., permethrins) (six compounds)	−1.65
Compounds containing a phenanthrene ring (four compounds)	+0.48
Multiply halogenated biphenyls and polyaromatics containing only aromatic carbons and halogens (e.g., PCBs) (19 compounds)	+0.62
Organometallic compounds containing tin or mercury (12 compounds)	+1.40

REFERENCE 18

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[Morimoto K et al; Drug Dev Ind Pharm 21 (17): 1999-2012 (1995)]**PEER REVIEWED**

Pharmacology:

Environmental Fate & Exposure:

Environmental Fate/Exposure Summary:

Capric acid's production and use as an ester for perfumes and fruit flavor, a base for wetting agents, an intermediate, a plasticizer, a resin, and as an intermediate for food-grade additives will result in its release to the environment from its use. **Capric acid** also occurs naturally in the environment as a component in glyceride oils. If released into the atmosphere, **capric acid** will exist solely in the vapor phase in the ambient atmosphere, based on a measured vapor pressure of 3.7X10-4 mm Hg at 25 deg C. Vapor-phase **capric acid** is degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals with a half-life of about 36 hours. An estimated Koc of 4,000 suggests that **capric acid** will have slight mobility in soil. Volatilization from moist soil is expected based upon an estimated Henry's Law constant of 1.3X10-6 atm-cu m/mole. Volatilization from dry soil surfaces should not be important given the vapor pressure of this compound. Aerobic biodegradation of **capric acid** is a relatively quick process, reaching an average theoretical BOD of 46 percent after 20 days in the presence of both sewage inoculum and activated sludge. In water, **capric acid** is expected to adsorb to sediment or particulate matter based on its Koc value. This compound is expected to volatilize from water surfaces given its estimated Henry's Law constant. Estimated half-lives from a model river and model lake are 36 days and 265 days, respectively. Bioconcentration in aquatic organisms should be high based upon an estimated BCF of 756. Given the commercial uses of **capric acid,** human exposure appears to be likely from occupational situations through dermal routes. The general population will be exposed to **capric acid** via ingestion of food, and dermal contact with vapors, food and other products containing **capric acid.** (SRC)
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Probable Routes of Human Exposure:

NIOSH (NOES Survey 1981-1983) has statistically estimated that 2,939 workers (276 of these are female) are potentially exposed to **capric acid** in the US(1). Occupational exposure may be through dermal contact with this compound at workplaces where **capric acid** is produced or used(SRC). The general population will be exposed to **capric acid** via inhalation of ambient air, ingestion of food and drinking water, and dermal contact with vapors, food and other products containing **capric acid**(SRC).

[(1) NIOSH; National Occupational Exposure Survey (NOES) (1983)]**PEER REVIEWED**

Natural Pollution Sources:

CAPRIC ACID, ISOLATED FROM AMERICAN ELM (ULMUS AMERICANA) SEEDS, WAS IDENTIFIED AS THE ANTIFUNGAL AGENT ACTIVE AGAINST THE DUTCH ELM DISEASE FUNGUS (CERATOCYSTIS ULMI) & SEVERAL OTHER FUNGI.
[DOSKOTCH RW ET AL; PHYTOPATHOLOGY 65(5) 634-5 (1975)]**PEER REVIEWED**

OCTANOIC ACID, **DECANOIC ACID,** DODECANOIC ACID, TETRADECANOIC ACID, & HEXADECANOIC ACID (11.0-18.7% OF THE TOTAL ACIDS) WERE ISOLATED FROM THE NATURAL SEX PHEROMONES OF MALE MEDITERRANIAN FRUIT FLY (CERATITIS CAPITATA).
[OHINATA K ET AL; J ENVIRON SCI HEALTH PART A A12(3) 67-78 (1977)]**PEER REVIEWED**

NATURAL FOOD OCCURANCES: ANISE, BUTTER ACIDS, OIL OF LIME, OIL OF LEMON.
[CHEMICALS USED IN FOOD PROCESSING; NAS/NRC PUBL 1274 WASHINGTON DC (1965)]**PEER REVIEWED**

CAPRIC ACID (0.9%) WAS FOUND IN THE SEED OIL OF GARCINIA MANGOSTANA.
[DAULATABAD CD, ANKALGI RF; J OIL TECHNOL ASSOC INDIA 10(2) 36-9 (1978)]**PEER REVIEWED**

Occurs as a glyceride in natural oils
[Hawley, G.G. The Condensed Chemical Dictionary. 10th ed. New York: Van Nostrand Reinhold Co., 1981. 190]**PEER REVIEWED**

Capric acid occurs as a component (along with caprylic acid and behenic acid) of caprenin, a triglyceride used as a low calorie cocoa butter substitute(1).
[(1) Friedman LJ et al; Kirk-Othmer Encycl Chem Tech. 4th ed. NY,NY: John Wiley and Sons 11: 815 (1994)]**PEER REVIEWED**

Artificial Pollution Sources:

Capric acid's production and use as an ester for perfumes and fruit flavor, a base for wetting agents, an intermediate, a plasticizer, a resin, and as an intermediate for food-grade additives(1) will result in its release to the environment(SRC).

[(1) Lewis RJ; Hawley's Condensed Chemical Dictionary. 12th Ed. NY,NY: Van Nostrand Reinhold Company p. 213(1993)]**PEER REVIEWED**

Environmental Fate:

TERRESTRIAL FATE: Based on a recommended classification scheme(1), an estimated Koc of 4,000(SRC), determined from a measured log Kow of 4.09(2) and a recommended regression-derived equation(4), indicates that **capric acid** is expected to have slight mobility in soil(SRC). Volatilization of **capric acid** from moist soil surfaces is expected(SRC) given an estimated Henry's Law constant of 1.3X10-6 atm-cu m/mole(SRC), determined from experimental water solubility(3) and vapor pressure(5). **Capric acid** is not expected to volatilize from dry soil surfaces based on an experimental vapor pressure of 3.7X10-4 mm Hg(5,SRC). Aerobic screening studies reveal that biodegradation of **capric acid** is a relatively quick process, reaching an average theoretical BOD of 46 percent after 20 days in the presence of both sewage inoculum and activated sludge(6,7,SRC). Therefore, biodegradation in soil is expected to be an important fate process(SRC).
[(1) Swann RL et al; Res Rev 85: 23 (1983) (2) Hansch C et al; Exploring QSAR. Hydrophobic, Electronic, And Steric Constants. ACS Prof Ref Book. Washington, DC: Amer Chem Soc p. 81 (1995) (3) Yalkowsky SH, Dannenfelser RM; The AQUASOL dATAbASE of Aqueous Solubility. Fifth ed, Tucson, AZ: Univ Az, College of Pharmacy (1992) (4) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington DC: Amer Chem Soc pp. 4-9 (1990) (5) Baccanari DP et al; Trans Faraday Soc 64: 1201-5 (1968) (6) Gaffney PE, Heukelekian H; J Water Pollut Control Fed 33: 1169-83 (1961) (7) Malaney GW, Gerhold RM; pp 249-57 in Proc 17th Ind Waste Conf, Purdue Univ, Ext Ser 112 (1962)]**PEER REVIEWED**

AQUATIC FATE: Based on a recommended classification scheme(1), an estimated Koc of 4,000(SRC), determined from a measured log Kow of 4.09(2) and a recommended regression-derived equation(4,SRC), indicates that **capric acid** is expected to adsorb to suspended solids and sediment in water(SRC). **Capric acid** is expected to volatilize from water surfaces(4,SRC) based on an estimated Henry's Law constant of 1.3X10-6 atm-cu m/mole(SRC), determined from its experimental vapor pressure of 3.7X10-4 mm Hg(5) and water solubility(3). Estimated volatilization half-lives for a model river and model lake are 36 days and 265 days, respectively(4,SRC). According to a classification scheme(6), an estimated BCF of 756(4,SRC), from the experimental log Kow(2,SRC), suggests that bioconcentration in aquatic organisms is high(SRC). Aerobic screening studies reveal that biodegradation of **capric acid** is a relatively quick process, reaching an average theoretical BOD of 46 percent after 20 days in the presence of both sewage inoculum and activated sludge(7,8,SRC).
[(1) Swann RL et al; Res Rev 85: 23 (1983) (2) Hansch C et al; Exploring QSAR. Hydrophobic, Electronic, And Steric Constants. ACS Prof Ref Book. Washington, DC: Amer Chem Soc p. 81 (1995) (3) Yalkowsky SH, Dannenfelser RM; The AQUASOL dATAbASE of Aqueous Solubility. Fifth ed, Tucson, AZ: Univ Az, College of Pharmacy (1992) (4) Lyman WJ et al; Handbook of Chemical Property Estimation Methods.

Washington DC: Amer Chem Soc pp. 4-9, 5-4, 5-10, 15-1 to 15-29 (1990) (5) Baccanari DP et al; Trans Faraday Soc 64: 1201-5 (1968) (6) Franke C et al; Chemosphere 29: 1501-14 (1994) (7) Gaffney PE, Heukelekian H; J Water Pollut Control Fed 33: 1169-83 (1961) (8) Malaney GW, Gerhold RM; pp. 249-257 in Proc 17th Ind Waste Conf, Purdue Univ, Ext Ser 112 (1962)]**PEER REVIEWED**

ATMOSPHERIC FATE: According to a model of gas/particle partitioning of semivolatile organic compounds in the atmosphere(1), **capric acid,** which has an experimental vapor pressure of 3.7X10-4 mm Hg at 25 deg C(2,SRC), is expected to exist solely as a vapor in the ambient atmosphere. Vapor-phase **capric acid** is degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals(SRC); the half-life for this reaction in air is estimated to be about 34 hours(3,SRC).
[(1) Bidleman TF; Environ Sci Technol 22: 361-367 (1988) (2) Baccanari DP et al; Trans Faraday Soc 64: 1201-5 (1968) (3) Meylan WM, Howard PH; Chemosphere 26: 2293-99 (1993)]**PEER REVIEWED**

Environmental Biodegradation:

The 5 day BOD of **capric acid,** concn 100 ppm, was determined to be 8.52 mmol/mmol **capric acid** using acclimated mixed microbial cultures in a mineral salt medium(1). **Capric acid,** present at 10,000 ppm, reached 45 to 53 percent and 46 to 54 percent of its theoretical BOD in 5 and 20 days, respectively, using a sewage inoculum(2). **Capric acid,** present at 10,000 ppm, reached 13, 45, and 46 percent of its theoretical BOD in 5, 10, and 20 days, respectively, using a sewage inoculum(3). In a similar study, **capric acid,** present at 10,000 ppm, reached 49, 53, and 54 percent of its theoretical BOD in 5, 10, and 20 days, respectively, using an acclimated sewage inoculum(3). **Capric acid,** present at unknown concn, reached 9 percent of its theoretical BOD in 5 days using a sewage inoculum(4). Using the Warburg test method, **capric acid,** present at 500 ppm, reached 29 to 42 percent of its theoretical BOD in 1 day, using an activated sludge inoculum with a microbial population of 2,500 mg/l corrected for endogenous respiration(5).
[(1) Babeu L, Vaishnav DD; J Indust Microbiol 2: 107-15 (1987) (2) Gaffney PE, Heukelekian H; J Water Pollut Control Fed 30: 673-79 (1958) (3) Gaffney PE, Heukelekian H; J Water Pollut Control Fed 33: 1169-83 (1961) (4) Dore M et al; Trib Cebedeau 28: 3-11 (1975) (5) Malaney GW, Gerhold RM; pp. 249-257 in Proc 17th Ind Waste Conf, Purdue Univ, Ext Ser 112 (1962)]**PEER REVIEWED**

Biodegradation of 100 ppm **capric acid** using the cultivation method was 100 percent in river water and 100 percent in sea water after 3 days(1). The percentage of theoretical oxygen demand for 500 mg/l **capric acid** was determined to be 10.9, 18.9, and 23.4 after 6, 12, and 24 hours of exposure to activated sludge solids at 2,500 mg/l in the Warburg respirometer(2). An aerobic biodegradation screening study of **capric acid,** based on BOD measurements, using a sewage inoculum and an unknown **capric acid** concn, indicated 23 percent BODT over a period of 20 days(3). The biodegradation of 100 mg/l

capric acid by non-acclimated activated sludge over an unspecified time period was determined to have 100 percent TOC removal(4).
[(1) Kondo M et al; Eisei Kagaku 34: 188-95 (1988) (2) Malaney GW, Gerhold RM; J Water Poll Control Fed 41: R18-R33 (1969) (3) Nieme GJ et al; Environ Toxicol Chem 6: 515-27 (1987) (4) Yonezawa Y et al; Kogai Shigen Kenkyusho Iho 12: 85-91 (1982)]**PEER REVIEWED**

Environmental Abiotic Degradation:

The rate constant for the vapor-phase reaction of **capric acid** with photochemically-produced hydroxyl radicals has been estimated as 1.12X10-11 cu cm/molecule-sec at 25 deg C(SRC) using a structure estimation method(1,SRC). This corresponds to an atmospheric half-life of about 34 hours at an atmospheric concn of 5X10+5 hydroxyl radicals per cu cm(1,SRC). **Capric acid** is not expected to undergo hydrolysis(SRC) or direct photolysis in the environment due to the lack of functional groups to hydrolyze(2) or absorb in the environmental spectrum.
[(1) Meylan WM, Howard PH; Chemosphere 26: 2293-99 (1993) (2) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington DC: Amer Chem Soc pp. 7-4, 7-5 (1990)]**PEER REVIEWED**

Environmental Bioconcentration:

An estimated BCF of 756 was calculated for **capric acid**(SRC), using an experimental log Kow of 4.09(1) and a recommended regression-derived equation(2,SRC). According to a classification scheme(3), this BCF value suggests that bioconcentration in aquatic organisms is high(SRC).
[(1) Hansch C et al; Exploring QSAR. Hydrophobic, Electronic, And Steric Constants. ACS Prof Ref Book. Washington, DC: Amer Chem Soc p. 81 (1995) (2) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington DC: Amer Chem Soc pp. 5-4, 5-10 (1990) (3) Franke C et al; Chemosphere 29: 1501-14 (1994)]**PEER REVIEWED**

Soil Adsorption/Mobility:

The Koc of **capric acid** is estimated as 4,000(SRC), using a measured log Kow of 4.09(1) and a regression-derived equation(2,SRC). According to a recommended classification scheme(3), this estimated Koc value suggests that **capric acid** is expected to have slight mobility in soil(SRC).
[(1) Hansch C et al; Exploring QSAR. Hydrophobic, Electronic, And Steric Constants. ACS Prof Ref Book. Washington, DC: American Chemical Society p. 81 (1995) (2) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington DC: Amer Chem Soc pp. 4-9 (1990) (3) Swann RL et al; Res Rev 85: 23 (1983)]**PEER REVIEWED**

Volatilization from Water/Soil:

The Henry's Law constant for **capric acid** is estimated as 1.3X10-6 atm-cu m/mole(SRC) from its experimental values for vapor pressure, 3.7X10-4 mm Hg(1), and water solubility, 61.8 mg/l(2). This value indicates that **capric acid** will volatilize from water surfaces(3,SRC). Based on this Henry's Law constant, the volatilization half-life from a model river (1 m deep, flowing 1 m/sec, wind velocity of 3 m/sec) is estimated as approximately 36 days(3,SRC). The volatilization half-life from a model lake (1 m deep, flowing 0.05 m/sec, wind velocity of 0.5 m/sec) is estimated as approximately 265 days(3,SRC). **Capric acid's** Henry's Law constant(1,2,SRC) indicates that volatilization from moist soil surfaces is expected(SRC). **Capric acid** is not expected to volatilize from dry soil surfaces based on a measured vapor pressure of 3.7X10-4 mm Hg(1).
[(1) Baccanari DP et al; Trans Faraday Soc 64: 1201-5 (1968) (2) Yalkowsky SH, Dannenfelser RM; The AQUASOL dATAbASE of Aqueous Solubility. Fifth ed, Tucson, AZ: Univ Az, College of Pharmacy (1992) (3) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington DC: Amer Chem Soc pp. 15-1 to 15-29 (1990)]**PEER REVIEWED**

Environmental Water Concentrations:

GROUNDWATER: **Capric acid** was identified in trench leachates from Maxey Flats (Morehead), KY disposal site, concn unknown and West Valley, NY disposal site, concn ranging from 0.87 to 2.6 mg/l(1). Groundwater samples taken from wells in the Besos basin, Northeast Spain were found to contain **capric acid** concns ranging from 42 to 75 ng/l(2).
[(1) Francis AJ et al; Nuclear Technology 50: 158-63 (1980) (2) Guardiola J et al; Water Supply 7: 11-16 (1989)]**PEER REVIEWED**

DRINKING WATER: Grab samples of raw and treated water were taken at waterworks treating lowland river water in the UK between March and December 1976. **Capric acid** was identified in the initial survey of raw and treated water, concn unknown. In the survey of treated water, **capric acid** was identified in groundwater (potentially polluted) and surface water (river) samples taken between February 1979 and June 1979, concns unknown(1). **Capric acid** has been quantitatively detected, concn not reported, in drinking water samples collected from Poplarville, MS on March 2, 1979; Cincinnati, OH on October 17, 1978; Cincinnati, OH on January 14, 1980; New Orleans, LA on January 14, 1976; Miami, FL on February 3, 1976; Philadelphia, PA on February 10, 1976; Ottumwa, IA on September 10, 1976 and Seattle, WA on November 5, 1976(2). **Capric acid** has been identified as an organic disinfection byproduct (DBP) at a pilot plant in Evansville, IN, concn not reported(3).
[(1) Fielding M et al; Organic Micropollutants in Drinking Water; TR-159. Medmenham: Eng Water Res Cent (1981) (2) Lucas SV; GC/MS Analysis of Organics in Drinking Water Concentrates and Advanced Waste Treatment Concentrates; Vol 1: Analysis

Results for 17 Drinking Water, 16 Advanced Waste Treatment, ad 3 Process Blank Concentrates. USEPA-600/1-84-020A (NTIS PB85-128221) Columbus, OH: Batelle Columbus Labs, Health Eff Res Lab (1984) (3) Richardson SD et al; Environ Sci Technol 28: 592-99 (1994)]**PEER REVIEWED**

Effluent Concentrations:

An average **capric acid** concn of 1,788 ng/ul was identified in an industrial wastewater survey in which samples collected between November 1, 1979 to November 1, 1981 were analyzed for organic pollutants other than Priority Pollutants(1). Oil shale retort water from Rundle, Australia was found to obtain **capric acid** at a concn of 45 mg/l(2). **Capric acid** was identified in vapor at a concn of 10 ng/cu-M and on particles with a concn of 20 ng/g emitted during combustion of coal at Ames power plant in Iowa(3). Two oil-shale retort water samples produced from January to May 1979 at the Occidental Oil Shale, Inc. facility at Logan Wash, CO were reported to have an average **capric acid** concn of 31 mg/l and a DOC of 22.0 mg/l(4). 63 effluent water samples from industrial sites in Ohio, West Virginia, Pennsylvania, New Jersey, New York, Louisiana, Kentucky, Delaware, and Texas were collected and analyzed for **capric acid.** Site 26 reported a **capric acid** concn between 10 to 100 ug/l. Site 31 reported a **capric acid** concn ranging from < 10 to 100 ug/l(5). **Capric acid** was identified as having a concn of 3 ppb in process water effluent samples (from in situ coal gasification) from Gilette, WY and 123 ppb from boiler blowdown water effluent samples (from in situ oil shale processing) from DeBeque, CO(6). **Capric acid** was identified in acid fractions of Iona Island Sewage Treatment Plant(British Columbia, Canada) sewage and sludge effluent, concns up to 30 ug/l(7). Secondary effluents from ten municipal and industrial wastewater treatment plants discharging into Illinois rivers were sampled; **capric acid** was identified in effluents from St. Charles Public Owned Treatment Works (POTW), Addison POTW, and Decatur POTW, concn unknown(8).
[(1) Bursey JT, Pellizzari ED; Analysis of Industrial Wastewater for Organic Pollutants in Consent Degree Survey Contract No 68-03-2867 Athens, GA: USEPA Environ Res Lab pp 167 (1982) (2) Dobson KR et al; Water Res 19: 849-56 (1985) (3) Junk GA et al; in ACS Symposium Ser 319 (Fossil Fuels Util): 109-319 (1986) (4) Leenheer JA et al; Environ Sci Technol 16: 714-23 (1982) (5) Perry DL et al; Identification of Organic Compounds in Industrial Effluent Discharges. USEPA-560/6-78-009 NTIS PB-2919000 Columbus, OH: Batelle Columbus Labs (1978) (6) Pellizari ED et al; in ASTM Spec Tech Publ, STP 686: 256-74 (1979) (7) Rogers IH et al; Water Poll Res J Canada 21: 187-204 (1986) (8) Ellis DD et al; Arch Environ Contam Toxicol 11: 373-82 (1982)]**PEER REVIEWED**

Fine aerosol emission rates for **capric acid** from noncatalyst automobiles, catalyst automobiles, and heavy-duty diesel trucks were determined to be 3.2, 72.7, and 77.4 ug/km, respectively(1). **Capric acid** was identified in tire wear particles, brake lining particles, and road dust particles at concns of 37.8, 18.4, and 55.4 ug/g of particle sample, respectively(2). Aerosol samples were collected systematically throughout a complete annual cycle (1982) at four urban sites in southern California. Ambient annual concns of

capric acid ranged from 1.3 to 3.1 ng/cu-m(3). **Capric acid** was found in fine particulate abrasion products from green leaves at a concn of 183.3 ug/g and from dead leaves at a concn of 133.0 ug/g; samples collected were from trees characteristic of the Los Angeles, CA area(4). Fine particle emission rates for **capric acid** from a natural gas-fired water heater and a natural gas-fired space heater were determined. A HEPA-filtered dilution air sample emission rate for **capric acid** was determined to be 2.9 pg/kJ; the emission rate for **capric acid** through the first filter was determined to be 119.5 pg/kJ; the emission rate for **capric acid** through the backup filter was determined to be 131.3 pg/kJ(5).
[(1) Rogge WF et al; Environ Sci Technol 27: 636-51 (1993) (2) Rogge WF et al; Environ Sci Technol 27: 1892-1904 (1993) (3) Rogge WF et al; Atmos Environ 27A: 1309-30 (1993) (4) Rogge WF et al; Environ Sci Technol 27: 2700-11 (1993) (5) Rogge WF et al; Environ Sci Technol 27: 2736-44 (1993)]**PEER REVIEWED**

Sediment/Soil Concentrations:

Sediment samples collected on September 28, 1990 from Dokai Bay in north Kyushu, Japan were found to contain **capric acid** at unknown concns(1).
[(1) Terashi A et al; Bull Environ Contam Toxicol 50: 348-55 (1993)]**PEER REVIEWED**

Atmospheric Concentrations:

URBAN/SUBURBAN: Sampling of particulate matter and gaseous pollutants was conducted for three weeks between October 7, 1976 and October 29, 1976 in Belgium; **capric acid** was identified in the gas phase of the urban air samples, concn unknown(1). RURAL/REMOTE: **Capric acid** was found on aerosols obtained over the southern North Atlantic Ocean with a mean concn of 4.9 ng/cu-m at STP(2). Analysis of the atmosphere in the Eggegebirge forest in North Rhine-Westflia, western Germany was found to contain **capric acid,** concns unknown(3). Aerosol samples were collected from a tower on Enewetak Atoll 1, Marshall Islands, a tower on the RV Moana Wave in the North Pacific Ocean and from American Somoa; **capric acid** concns were determined to be 0.11, 0.025, and 0.49 to 3.7 ng/cu-m, respectively(4). RAIN/SNOW: **Capric acid** has been identified in rain/snow: aqueous (wet-only) from rural Hubbard Brook, NH and semi-rural Ithaca, NY; samples collected between June 1976 and May 1977 were determined to have an average **capric acid** concn of < 0.1 umol/75 cm precipitate(5).
[(1) Cautreels W, VanCauwenberghe K; Atmos Environ 12: 1133-41 (1978) (2) Duce RA et al; Rev Geophysics Space Physics 21: 921-52 (1983) (3) Helmig D et al; Chemosphere 19: 1399-1412 (1989) (4) Kawamura K, Gagosian RB; Nature 325: 330-32 (1987) (5) Mazurek MA, Simoneit BRT; CRC Critical Reviews in Environmental Control 16: 1-140 (1986)]**PEER REVIEWED**

Food Survey Values: